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RELAPSING FEVER: SOME RECENT ADVANCES.

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The present article comprises references to some of the more important recent advances in our knowledge of human relapsing fevers. With few exceptions attention will be confined to papers that have appeared during the last five years, since the writer's article on blood spirochaetes (HINDLE, 1931), contains a brief summary of our knowledge of the subject up to 1930, based on publications previous to that date.

Epidemiology.

In recent times there has been a remarkable diminution in the number of cases of relapsing fever in Europe and North Africa, but in 1930, endemic foci still persisted in Italy and especially Russia (League of Nations Report, 1930). Contrasting with this post-war decline in European countries, an extensive and deadly epidemic of louse-borne relapsing fever swept across Equatorial Africa, starting from Upper Guinea about 1921. According to LASNET (1930) the disease was probably introduced by natives from the Mediterranean region, as the first cases at Kouroussa occurred among Moroccan and Algerian soldiers. It spread down the Niger and during 1922 epidemics occurred towards the east in the Dori region, and the number of deaths caused during the first two years in the French Sudan and Niger is estimated at 80,000 to 100,000. In 1924 it spread to the Upper Volta region and across to Koutiala, causing at least 20,000 deaths. In 1925, it broke out in the Lake Chad region and invaded Northern Nigeria and the Cameroons. It persisted until 1929 in North Equatorial Africa and about 10 per cent. of the population is estimated to have died of the disease, the mortality varying from 5 to 25 per cent. of the whole population. The epidemic reached Darfur in September 1926, and according to ATKEY (1929) in one district alone 10,000 died out of a total population of 45,000. It was brought under control in 1928, although subsequently isolated outbreaks have occurred from time to

time. Apart from this great epidemic, a number of smaller outbreaks have been recorded in other parts of the world, among the more interesting being sporadic cases of tick-transmitted relapsing fever in North America (see below).

The following list comprises a brief summary of publications during the past five years dealing with cases of relapsing fever arranged according to the countries in which they occurred.

Europe.—SYSSINE (1931) records a steady diminution of the disease in Russia, from 19,701 cases in 1925 to only 1,656 in 1930.

Asia.—SACHS (1934), in a detailed analysis of relapsing fever in Chitral during 1932 and 1933, considers that the clinical evidence supports the view that both tick and louse-borne varieties were present. TSCHIREJKIN (1930) describes 10 cases of Bokharan relapsing fever; KATZ (1930) gives an account of 38 cases of the "Persian variety" observed in the Western Pamirs, and KASSIRSKY (1933) a description of the main features of Central Asiatic tick fever based on a study of 78 cases at TASHKENT. ROBERTSON (1932) contributes a general discussion of relapsing fever in China, with special reference to Shanghai, where the clinical symptoms resemble those of the European strain. CHU, DEITRICK and CHUNG (1931) give the results of a study of 26 cases in children in Peking, and HIROKI (1933) isolated strains of Manchurian relapsing fever and studied them with special reference to the persistence of residual brain infections. TOYODA (1931) gives a general review of the subject with special reference to the Manchurian strain.

Africa.—Numerous papers have been published on the various strains of relapsing fever occurring in North Africa and their relation to the infections occurring in wild rodents. In Morocco, DELANOË (1929) considers that at least three strains may occur, *S. recurrentis*, the ordinary European form, *S. hispanica*, or a related form, causing the Spanish-African type of relapsing fever, and *S. marocana*, causing a mild non-relapsing type. On the other hand, NICOLLE and ANDERSON (1929b) find that all the strains of tick-transmitted relapsing fever in Morocco belong to the same species, *S. hispanica*. This Spanish-African strain, variously referred to as either *S. hispanica*, or *S. hispanica* var. *marocana*, has now been recorded from Algeria, by SERGENT (A.), MANCEAUX and BALLISTE (1933), and HORRENBERGER (1933). In Tunis, NICOLLE, ANDERSON and LE CHUITON (1931) observed three cases of the same strain, which were also studied by NICOLLE, ANDERSON and LAIGRET (1932) and two further cases by NICOLLE, LAIGRET and SICARD (1933). What seems to be a new strain of the *hispanica* group, has been isolated by ANDERSON and WASSILIEFF (1933) from *Ornithodorus erraticus* collected from burrows of *Meriones shawi* in South Tunisia. In Cyrenaica (Tripoli) cases of relapsing fever, probably tick-transmitted, have been recorded by FRANCHINI and TADDIA (1930) and MEDULLA (1931). A focal epidemic in Asmara, Eritrea, is supposed by DE PAOLI (1930) to have been introduced by travellers from Abyssinia.

Further South reference has already been made to the great epidemic of louse-borne relapsing fever which devastated North Equatorial Africa from 1921 to 1929, described by LASNET (1930), CAZANOVE (1930) and LE GAC (1931). Also a small epidemic occurred during 1927 and 1928 in villages of the Dori region (MALTZER, 1929). RUSSELL (1931), contributed a valuable study of cases occurring in the Gold Coast during 1929 and 1930.

Our knowledge of tick-transmitted relapsing fevers in West Africa, especially at Dakar, is summarized by MATHIS (1931). The discovery of *Ornithodorus erraticus* in that region by DURIEUX (1932) removed the problem surrounding the transmission of the local strain of *S. duttoni* (=*S. duttoni* var. *crocidurae*), for until then no one had succeeded in finding Ornithodorus in Senegal. MATHIS and DURIEUX (1934a) and FEYTE (1932) have brought forward evidence in support of the view that the disease is probably much more common in Dakar than the number of recorded cases would lead one to suppose.

ADVIER, ALAIN and RIOU (1934) have given a general account of cases observed in Dakar and other parts of Senegal, and call attention to the difficulties of diagnosis, as spirochaetes were found in the blood of only 25 out of 46 patients, and even then were extremely rare. MATHIS and DURIEUX (1934b) demonstrated the existence of an endemic centre at St. Louis, Senegal, whilst DUBOIS (1931a) compared two strains of *S. duttoni* from different parts of the Congo, and found that they were immunologically distinct.

America.—Although previously suspected, the first definite record of the existence of tick-transmitted relapsing fever in the U.S.A. has been made by WELLER and GRAHAM (1930), who traced cases of the infection in Central Texas to a cave in the Colorado River Valley, containing large numbers of *Ornithodorus turicata*, the transmitting agent. Subsequently sporadic cases have been recorded from various other parts of the Southern United States. PORTER, BECK and STEVENS (1932) give a useful summary of 30 cases in California, where there is good evidence that wild rodents harbour the infection. (See also LEGGE, 1933). In addition, PALMER and CRAWFORD (1933) give details of six cases occurring in British Columbia, the first record of relapsing fever in Canada, where the wood tick, *Dermacentor andersoni*, is considered to be the most probable vector.

Relapsing fever in California has been the subject of a detailed account by COLEMAN (1933 and 1934 a & b), based on the study of three strains isolated from human cases. These strains were found to differ from *S. duttoni* and *S. novyi*, as judged by cross-immunity tests. Relapsing fever in Texas has been studied by KEMP, MOURSUND and WRIGHT (1933), who found that it was immunologically identical with *S. novyi*. Subsequently (1934), these authors made transmission experiments with *Ornithodorus turicata*, comparing the Texas strain with four other strains, *S. novyi*, *S. kochi*, *S. duttoni* and *S. recurrentis*. In feeding experiments none of these four strains could be transferred from rat to rat by *O. turicata*, and the spirochaetes died out in the tick within a week of being ingested. BRUMPT (1933) on the basis of similar results, named the causative organism *Spirochaeta turicatae*, as it differs from *S. novyi* in being transmissible by *O. turicata*.

DUNN and CLARK (1933) give a general account of relapsing fever in Panama, where it is known to have been endemic since 1905. *Ornithodorus talaje* and *O. venezuelensis* are prevalent and both have been shown to be efficient carriers.

Animal Reservoirs.

Since NICOLLE and ANDERSON (1927) developed the interesting hypothesis that small mammals, and especially rodents, commonly serve as reservoirs of infection for relapsing fever, there has been abundant evidence in support of their view in the large number of wild rodents that have been found naturally infected with various strains of

spirochaetes, pathogenic to man. In addition, collections made from the burrows of rodents have often revealed the presence of infected ticks, which had previously escaped notice. As might have been expected, much of the work on this subject has been conducted by NICOLLE and his colleagues, working in North Africa, where strains of relapsing fever have been isolated from a number of animal sources.

DELANOË (1929) isolated a strain of Moroccan relapsing fever (*S. hispanica* var. *marocana*) from *Ornithodorus* collected from the burrows of porcupines in Morocco, and later (1930) from the porcupines themselves. The strain was pathogenic to guineapigs and three men inoculated with infected blood from these animals showed scanty spirochaetes from the 8th to the 10th day. NICOLLE, ANDERSON and COLAS-BELCOUR (1929) found that a young porcupine could be infected with this strain and its blood became infective to other animals, but in view of the comparative rarity of the porcupine and its habitat, it is not considered to be as likely a host as the common small rodents.

Algerian foxes have also been found naturally infected with the Moroccan strain (DELANOË, 1931a) and infected *Ornithodorus* have been collected from their burrows. Spontaneous infections with the same strain also occur in the jackal and hedgehog (DELANOË, 1931b). According to BLANC, NOURY and FISCHER (1933), another important reservoir of this strain is the common grey rat, *Mus norvegicus*, for at Casablanca at least 1 in 22 was infected, as tested by the inoculation of their brains into guineapigs. DELANOË (1933b) also found a young weasel, *Putorius vulgaris*, caught in Morocco, naturally infected with *S. hispanica*. The central nervous system of this animal remained infective after the blood had become negative. The examination of large numbers of wild animals in Morocco (DELANOË, 1931c), indicates that infection with this strain of relapsing fever is very widespread in nature and there is no likelihood of its being eradicated.

A strain isolated from Getulie squirrels (*Atlantoxerus getulus*) by BLANC, NOURY, BALTAZARD and FISCHER (1933), is said to resemble the ordinary Spanish-African type; but DELANOË (1933a), in specimens collected at Agadir, found a blood spirochaete differing considerably from the typical *S. hispanica*, for guineapigs were refractory, as well as *Meriones*. Moreover, experimentally this worker failed to infect squirrels by subcutaneous inoculation of *S. hispanica*, and considers that it is not likely to be a reservoir.

ANDERSON and WASSILIEFF (1933) obtained a new strain of relapsing fever from *Ornithodorus erraticus* collected from the burrows of *Meriones shawi* in South Tunis. Porcupines inoculated with the strain had a short infection with visible spirochaetes; *Meriones*, a non-apparent infection, with no visible spirochaetes; and two human subjects had severe attacks, one of them being fatal.

A. SERGENT (1933) studying the locality of the first Algerian case of Spanish-African relapsing fever, found *Rhipicephalus sanguineus*, from the patient's dog, naturally infected with the spirochaete, as tested by the inoculation of these ticks into guineapigs. Moreover, this species was subsequently shown to be capable of transmitting the infection by its bite, so that Spanish-African relapsing fever can be spread not only by *Ornithodorus* but also by the dog tick.

Batches of ticks, *O. erraticus*, collected in or near Dakar from burrows of rats, were found by DURIEUX (1932) to be naturally infected with the local strain of relapsing fever, as tested by feeding experiments on 10

patients (MATHIS, DURIEUX and ADVIER, 1933, 1934). An examination of the local fauna by MATHIS and DURIEUX (1934a) shows that many of the rodents can serve as reservoirs of infection in addition to *Crocidura stampflii*, which is the most important. As tested by the inoculation of blood or brain emulsion into mice or rats, the following species were found to harbour the infection :—*Epimys decumanus*, *E. alexandrinus*, *E. rattus*, *E. golonda campanae*, *E. coucha*, and the common wild mice *Mus musculus gentilis* and *M. m. spretus*. The two latter, however, were only rarely infected. Similar experiments, also by MATHIS and DURIEUX (1934b), showed that wild rats, *Epimys decumanus* and *E. golonda campanae*, collected in St. Louis, Senegal, and the neighbourhood, were naturally infected with the same strain of relapsing fever. ADANT (1932) examined wild rodents from various parts of Katanga Province and found a wild rat, *Aethomys kaiseri*, infected with a strain of relapsing fever pathogenic to man, but not to guinea-pigs, thereby differing from the Spanish-African strain.

In California, PORTER, BECK and STEVENS (1932) obtained the infection from nine chipmunks and two squirrels, by the inoculation of their blood into white mice, and further support to the view that field rodents are victims of spirochaetes which may be conveyed to man, is afforded by the record of a case in Sierra County, where the patient, a medical entomologist, was infected by the contamination of a wound with the blood of a freshly killed tamarack squirrel, which was subsequently found to contain spirochaetes (LEGGE, 1933).

In Panama, an extraordinarily wide range of animals has been found to harbour spirochaetes which seem to be identical with the human infection, including marmoset monkeys (*Leontocebus geoffroyi*), opossums (*Didelphis marsupialis etensis*), armadillos (*Dasypus novemcinctus fenestratus*), calves and a horse (DUNN and CLARK, 1933). It would seem, therefore, that in the case of tick-transmitted relapsing fevers, a wide range of animals may serve as reservoirs for the infection, and the importance of any species in this connexion probably depends to a large extent on its numbers and habitat, and especially on its proximity to human habitations. Consequently, the smaller rodents are likely to be the most important carriers.

Transmission.

In nature, all known varieties of relapsing fever are transmitted exclusively by the agency of lice, ticks, especially those belonging to the genus *Ornithodorus*, and possibly of bed-bugs. With reference to the latter, recent observations tend to show that the bed-bug is a more favourable host than was previously suspected, and although epidemiological evidence does not support the view that they are of any great importance as carriers, yet their possibilities cannot be entirely ignored. ROSENHOLZ (1927) in a careful series of experiments found that when spirochaetes were ingested by bugs the organisms invaded the haemocoele and persisted there indefinitely, although they gradually disappeared from the gut. These spirochaetes in the haemocoele retained their virulence and presumably could reproduce the infection in human beings in the same way as the body-louse, by the infected contents getting on to an excoriated surface, since the mere bite of these infected bed-bugs was quite innocuous. CZARKOWSKA and BLANK-WEISSBEG (1930) found that spirochaetes persisted in the gut of the bed-bug up to 46 days after an infective feed, but became motionless after 48 hours. Active spirochaetes appeared in the haemolymph after 6 to 7 days and

the injection of infected bugs up to the 15th day, and also of haemolymph, produced infection in mice. KLEINE and KRAUSE (1934) found that when larvae of bed-bugs were fed on mice infected with both European and African strains of relapsing fever, about 4 per cent. remained infective up to 80 days after the infective meal. On the other hand 150 adult bugs similarly fed gave uniformly negative results, and also 3,000 larvae reared from infected parents were inoculated into mice without producing any infection. It is concluded, therefore, that these insects can only play a very small part as carriers of relapsing fever, not to be compared with that of ticks or lice.

Many species of *Ornithodorus* have been found naturally infected with spirochaetes infective to man, and NICOLLE and ANDERSON (1929a, 1929c) give further experiments in support of their view that any species of *Ornithodorus* is capable of transmitting all strains of relapsing fever normally transmitted by ticks belonging to this genus. They insist, however, that it is necessary to feed the ticks on the infected animal during the nymphal stage in order to succeed. DELANOË (1931c) also gives two examples of unsuccessful attempts to infect adult *Ornithodorus erraticus* with the Moroccan strain of relapsing fever. These conclusions are opposed by KRITSCHEWSKI and DVOLAITSKAYA-BARISCHEWA (1931) who transmitted both *S. recurrentis* and *S. hispanica* (Berbera strain) by the bites of *Ornithodorus papillipes* infected only in the adult stage ; also by KLEINE and KRAUSE (1932b), who found that adult *Ornithodorus moubata* when fed on mice infected with *S. duttoni* became infective.

The discovery of *Ornithodorus erraticus* in Senegal by DURIEUX (1932) considerably extends the range of this important North African carrier, which has repeatedly been found infected with various strains of relapsing fever, even when collected from the burrows of animals far removed from human habitations. Among localities from which ticks infected with *S. hispanica* have been found may be mentioned pigsties, the burrows of porcupines and fox-holes (DELANOË, 1929), as well as the burrows of small rodents. Moreover, in addition to *S. hispanica*, this species of tick has been shown to be the natural carrier of the following strains :—*S. normandi* (NICOLLE, ANDERSON and COLAS-BELCOUR, 1929) ; a strain found at Carthage related to the latter, but referred to as *S. erratici* (NICOLLE, ANDERSON and LAIGRET, 1932) ; another human strain isolated by ANDERSON and WASSILIEFF (1933) from ticks collected from burrows of *Meriones shawi* in South Tunis ; whilst in Dakar it takes the place of *O. moubata* as the carrier of the local relapsing fever, the agent of which was previously named *S. crocidurae*, but is now considered identical with *S. duttoni* (MATHIS, DURIEUX and ADVIER, 1933). This species also transmits a group of spirochaetes found in small rodents, including *S. gondii*, which is very feebly pathogenic to guineapigs and rats, and does not infect man (NICOLLE and ANDERSON, 1930).

Ornithodorus papillipes.—Successful transmission experiments with this species, using the tick-borne Central Asiatic strain of relapsing fever (*S. persica*, *S. sogdiana*, or *S. usbekistanica*), have been recorded by MOSKWIN (1929), KRITSCHEWSKI and DVOLAITSKAYA-BARISCHEWA (1931), and PAVLOVSKIĬ (1932). The latter worker failed to transmit the disease by *Ornithodorus lahorensis* and considers that *O. papillipes* is the only proved vector in Central Asia.

Ornithodorus turicata.—This widely distributed species seems to be the most important carrier of tick-transmitted relapsing fever in the

U.S.A. WELLER and GRAHAM (1930) traced cases of relapsing fever in Texas to a cave containing large numbers of these ticks. BRUMPT (1933) found that the Texas strains were readily transmitted to mice, rats, and *Peromyscus* by the bites of *O. turicata*, but all attempts to transmit *S. hispanica*, *S. duttoni* and *S. venezuelensis*, and also *S. novyi* (BRUMPT, 1934a), gave negative results. On the other hand, NICOLLE, ANDERSON and LAIGRET (1932) recorded the experimental transmission of a strain of *S. hispanica* found in Tunis, by the bites of *O. turicata*. The infection was by bite from one nymphal stage to the next. KEMP, MOURSUND and WRIGHT (1934) also obtained similar results, and working with *S. novyi*, *S. kochi*, *S. duttoni* and *S. recurrentis*, found that none of these four strains could be transmitted from rat to rat by *O. turicata*; moreover, the spirochaetes died out in the tick within a week of being ingested. The Texas strain, however, was easily transmitted by its bite, rabbits, monkeys and rats being infected. Although they state that hereditary infection has been recorded, it is curious that these authors failed to infect rats by the inoculation of saline suspensions of eggs laid by infected ticks.

Ornithodorus venezuelensis has been shown capable of transmitting a spirochaete occurring naturally in the blood of squirrel monkeys, *Leontocebus*, caught in the Republic of Panama (CLARK, DUNN and BENAVIDES, 1931). One man was infected by the bites of nymphal and adult ticks fed on an infected monkey about five weeks previously. A batch of 60 larval ticks, reared from adults that had fed on an infected monkey, failed to produce any infection when allowed to bite a human volunteer.

Ornithodorus talaje, another carrier of human relapsing fever in Central America, has been found infesting opossums in Panama, of which 10 per cent. showed spirochaetes in their blood, transmissible to marmosets, rats and mice (DUNN and CLARK, 1933).

The possibility of ticks belonging to genera other than *Ornithodorus*, serving as carriers of human strains of relapsing fever is supported by the observations of A. SERGENT (1933), who in Algeria found specimens of *Rhipicephalus sanguineus* from a dog naturally infected with a strain of *S. hispanica*. Larvae of this dog tick were fed on infected guineapigs and after moulting the nymphs were allowed to gorge on four normal guineapigs. After 17 days interval, one of these animals became infected, with numerous spirochaetes in its blood. In addition, PALMER and CRAWFORD (1933), consider that the wood tick, *Dermacentor andersoni*, is the most probable vector of cases of relapsing fever occurring in the West Kootenay district of British Columbia. LEGGE (1933) found only *Ixodes* on Californian field rodents naturally infected with a strain of relapsing fever.

The problem of what happens to the spirochaete after being ingested by its transmitting host has been rendered still more uncertain by recent publications. KLEINE and KRAUSE (1932a) fed "clean" *Ornithodorus moubata* on a mouse infected with *S. duttoni* and found that spirochaetes, as such, persisted in the ticks for at least 33 days, and also remained infective. In some of the ticks, however, the spirochaetes died out. On the contrary, MOSKWIN (1929), working with "clean" *Ornithodorus papillipes* and a strain of Bokharan relapsing fever, found that within 12 days of being ingested, spirochaetes had completely disappeared from all parts of the tick. Yet these ticks were infective up to 170 days after the infective meal. This author traced all stages from the spirochaete in the alimentary canal to granular and cyst-like

forms in the Malpighian tubules, salivary glands and ovaries, and the inoculation of these organs into guineapigs in all cases produced infection. HATT (1929) by a study of sections of *Ornithodorus moubata* infected with *S. duttoni*, found that spirochaetes entered the cells of the tick and segmented into coccoid or bacillary forms, from which short spirochaetes subsequently developed. In this particular example the spirochaetes completely disappeared within 3 days of ingestion, but in the case of *S. hispanica* in *O. savignyi* the spirochaetes were still segmenting on the 5th day, had disappeared by the 9th day, and the coelomic fluid remained negative till the 25th day, when it became strongly positive, with all stages from short forms up to complete spirochaetes. Similar results were obtained with *O. savignyi* infected respectively with *S. duttoni* and *S. normandi*.

An entirely different life-cycle for *S. anserina*, the common fowl spirochaete, has been advanced by KNOWLES, GUPTA and BASU (1927). As a result of incessant division in the gut of the tick the spirochaetes are said to become about one-third the length of the ordinary blood forms, and extremely slender. These so-called "*tenue*" forms then invade the coelomic fluid and when there generally show a very fine terminal flagellum at each extremity. These forms are found in all parts of the body but accumulate especially in the salivary glands, where they develop into normal spirochaetes. Finally, MARCHOUX and CHORINE (1930) as a result of the examination of the blood of fowls bitten by infected *Argas*, concluded that there is an invisible infective stage of the spirochaete in the blood before the appearance of spiral forms.

Chemotherapy.

Relapsing fevers are generally treated by injections of salvarsan, or one of its derivatives, but all strains do not respond satisfactorily (*e.g.*, DICKINSON, 1932; DE LA CAMARA, FERNANDEZ MARTINEZ, DE BUEN and JUAREZ, 1932), and many attempts have been made to find more efficient therapeutic agents.

The method of administration is of importance, for KATZ (1930), in cases of tick-transmitted relapsing fever in the Pamirs, found that irregular doses of neosalvarsan merely prolonged the apyretic period without any appreciable alleviation of the symptoms. This author recommends three injections :—0·3 gm. on the first day of the attack; 3–4 days later 0·45 gm.; and finally, after 5–6 days interval, 0·6 gm.

ROSKIN and LEVINSON (1930) found that exposure to ultraviolet rays greatly increased the therapeutic and sterilizing action of salvarsan in mice infected with *S. duttoni*; 35 out of 47 irradiated mice were cured by one injection of salvarsan, as compared with only one out of 41 controls not exposed to the action of the lamp. Raising the body temperature, by keeping animals at 40°C. for two hours before and after an injection of salvarsan, has been found to have a very marked effect on residual infections (LEBEDEWA and GALANOWA, 1932). A number of mice infected with *S. duttoni* were treated in this way and 17 days later tested for sterility; all were found to have been completely sterilized, whilst out of the same number of controls treated at ordinary temperatures, 60 per cent. showed residual brain infections. An analysis of the organs of "heated" and control mice, showed that the brains of the former contained 5 to 10 times as much arsenic as those of "unheated" mice that had received the same dose of salvarsan. MENK (1931) recommends the use of a mixture of neosalvarsan and solganal, a gold preparation, which, in mice infected with *S.*

duttoni, was found to be approximately four times as effective as either of the drugs by itself.

For the treatment of human cases of tick fever in Nyasaland, J. TODD (1930) recommends the use of intramuscular injections of sodium potassium bismuth tartrate; for adults 0·2 gm. of the drug dissolved in 2 cc. sterile water on two successive days. Apart from being less expensive, the drug is said to be much more effective than novarsenobenzol, since the temperature is brought down within 36 hours and relapses are almost unknown.

HASSKÓ (1933) tested the effect of 15 trivalent and 5 pentavalent arsenic compounds on mice infected with *S. duttoni* and found only one (BR 34) to give results comparable with neosalvarsan. Antimony in the form of Stibosan H471, was found to be effective in the treatment of infections with Central Asiatic tick fever. Bismuth-yatren A has been used with success in the treatment of mice infected with *S. recurrentis* (KRITSCHEWSKI, 1930), and many gold preparations have been tested with more or less favourable results, especially in the treatment of residual brain infections, or strains resistant to arsenical compounds. Among the gold salts that have been recommended may be mentioned Sulpho-crisolo I.S.M. (CUBONI, 1929a); Allochrysine (HOWARD, 1929); Triphal (BASKIN, 1931); Solganal, or Solganal B (DUBOIS, 1931b); and various gold compounds, of which Solganal gave the best results (TODA, 1931).

The method of action of drugs in the treatment of spirochaetal infections has been studied by various authors, who disagree in their conclusions. MORETTI (1929) in the case of arsenical compounds, found that splenectomy, or blocking the reticulo-endothelial system, greatly reduced, if it did not entirely abrogate, the effect of the drug; hence the integrity of this system is considered to be essential for successful chemotherapy. HASSKÓ (1932), as the result of experiments with *S. recurrentis* in mice, came to the conclusion that this infection has a transitory paralysing effect on the reticulo-endothelial system, and suggests that drugs are first absorbed by the spirochaetes, and subsequently deposited in the tissues together with them.

STERNBERG and PINES (1933) concluded that in the case of Stibosan the drug acted directly on the spirochaete and not through the tissues of the host.

An interesting new method of studying these problems has been introduced by SINGER and FISCHL (1934) and FISCHL, KOTRBA and SINGER (1934), who, by means of chemical analysis of the mineral content of the spirochaetes, determine the amount of arsenic or gold present in these organisms before and after treatment with organic arsenical or gold preparations. Their results suggest that the chemotherapeutic agents unite directly with the spirochaetes, but further *in vitro* experiments led SINGER, KOTRBA and FISCHL (1934) to the view that the action is a complex phenomenon comprising three phases:—(1) A physico-chemical adsorption of the substance by the pathogenic organism; (2) a change in this adsorbed substance owing to the vital activities of the cell, resulting in the formation of an actual poison; and (3) the completion of cure by the immune substances of the organism of the host. FELDT (1934) used the same method in a study of the action of chemotherapeutic substances in rats infected with three strains of relapsing fever and spirochaetes; one normal, another resistant against salvarsan, and the other against solganal. It was found that the resistant strains contained approximately the

same (never less) quantities of arsenic or gold as the normal strain, a result which supports the view that salvarsan and solganal do not act directly on the parasites. The action of these compounds is supposed to be through the natural defence mechanism of the animal body, and the resistance of spirochaetes to be dependent on their resistance to this defence function. This view is also supported by the results of KROÓ (1934), who found that, in spite of the favourable chemotherapeutic index of arsenobenzol in the treatment of fowl spirochaetosis, one-day old chicks inoculated with the maximum tolerated dose were not cured, for after a time spirochaetes reappeared in their blood.

A summary of previous work on *S. duttoni* is given by GRAY (1929) who found that his strain was markedly resistant to organic arsenicals; also a bismuth compound, "Bismostab," had little or no effect on the disease. CUBONI (1929a), using an arsenic resistant strain of *S. duttoni* in mice, found that the infection could be cured by Sulfo-crisolo I.S.M., a gold preparation. FELDT (1932) succeeded in producing strains of *S. recurrentis* resistant against salvarsan and solganal, respectively. The salvarsan-resistant strain, after passaging in normal mice for 10 weeks lost its resistance, but the solganal strain was still resistant after 19 months. Resistant strains of *S. pallida* were also produced. According to KRITSCHEWSKI and DEMIDOWA (1932), when salvarsan-resistant strains of *S. duttoni* in mice are exposed to the action of sodium thiosulphate (0·05 cc. of a 2·5 per cent. solution per gm. body weight), they become susceptible to the action of salvarsan, in contrast with the original strains which retain their resistance. The alteration is supposed to be the result of new chemoreceptors being formed on the spirochaetes by the action of the thiosulphate.

Two strains of Manchurian relapsing fever, one producing residual brain infections and the other not, were found by TODA (1931) to be identical in their resistance to neosalvarsan. ROTHERMUNDT (1932), also with WICHMANN (1932), as the result of testing the effect of a number of chemotherapeutic substances, including arsenic, antimony, bismuth and gold compounds, on mice infected with *S. duttoni*, came to the conclusion that the examination of the brain for spirochaetes as a criterion of the efficiency of any chemotherapeutic substance, and also the use of persistent brain infections for general chemotherapeutic experiments, are of little value.

General Pathology.

The changes in the spleen histology of 15 fatal cases of relapsing fever in the Gold Coast have been described by RUSSELL (1932), who also gives a useful historical survey of the subject. The most important and characteristic change was found to be the occurrence of miliary lesions, consisting of a zone of congestion and cell infiltration round the Malpighian bodies. BRUMPT (1934b) found that guineapigs infected with a Central Asiatic strain, transmitted by *O. papillipes*, often showed a large clot apparently arising from the anterior end of the spleen, and the peritoneum contained a quantity of non-coagulated blood. A study of the effects of three strains of relapsing fever spirochaetes in the eyes of rabbits is given by IGERSHEIMER and BODENHEIMER (1928), who found that the spirochaetes may persist in the cornea after their disappearance from other parts of the body.

Changes in the leucocyte formula in relapsing fever are recorded by MURATET and LE GAC (1930), who studied cases at Wadai, Central

Africa. Although the number of polymorphonuclears and mononuclears did not alter appreciably, a more detailed examination showed that the number of lymphocytes diminished whilst the mononuclears increased, and the polymorphonuclears increased at the expense of the eosinophiles. In addition, the Arneth index was always deviated towards the left. CHU, DEITRICK and CHUNG (1931), in children infected with relapsing fever in China, found the leucocyte count of little value, but thrombocytopenia was constantly observed in the febrile attacks, the number of platelets falling below 100,000, returning after recovery to 200,000 to 300,000. No prolongation of the bleeding or coagulaton time was noticed. An examination of the blood of guineapigs infected with *S. hispanica*, by VAN DEN BRANDEN, DUMONT and NELIS (1930), showed that the blood sugar content remained unaffected by the presence of this spirochaete. The urine of guineapigs infected with *S. hispanica* was found to contain the virus at the height of the infection in 2 out of 6 animals; also the aqueous humour, but the vitreous humour was always negative (REMLINGER and BAILLY, 1929b).

IMMUNITY.

The discovery of soluble specific substances in spirochaetes (HINDLE and Bruce WHITE, 1934), has introduced a new method for the study of spirochaetal immunity. The isolation of these substances in each case has been effected by solution of the spirochaete in 0·5 per cent. NaOH, followed by extraction with industrial alcohol, treatment of the filtrate with acetic acid, then refiltration, and further precipitation of the filtrate with acetone. By a process of differential precipitation an acetone-insoluble fraction was obtained free from protein, which is considered to be either a carbohydrate or a carbohydrate-containing substance. It reacted in high dilutions with homologous antisera, producing a zone of precipitation at the junction of the solution and antiserum. Since repeated injections of the substance into normal rabbits failed to produce any precipitating antibodies, it is considered to fall into the category of haptenes.

In relapsing fevers the study of immunity is complicated by the fact that the spirochaetes of successive attacks often differ in their serological characteristics. A detailed investigation into the types of spirochaetes found in experimental infections with Indian relapsing fever, by CUNNINGHAM, THEODORE and FRASER (1934), shows that although in successive febrile attacks there is a tendency for the alternation of two main types, in addition, other types may develop following the first attack, or the first relapse. RUSSELL (1933) using African pouched rats, *Cricetomys gambianus*, infected with a strain of relapsing fever isolated in Accra, showed that the relapse strain ("B") was serologically distinct from that of the first attack ("A"). In successive passages if a rat infected with type B relapsed, it produced spirochaetes of type A, and conversely. In addition, a third type appeared in one of the second relapses. This author emphasized the importance of discounting results obtained with first passage animals when isolating strains of the relapse type, since, in addition to inoculating spirochaetes of this type, one also inoculates immune bodies against spirochaetes of the first attack, and the passive immunity thereby induced is liable to influence the type with which the animal is expected to relapse. JAKIMOW (1929) found that in a case of a Berlin strain of

S. recurrentis, the relapse strain was distinct from the original strain in about 50 per cent. of the cases tested ; whilst GRAY (1929), in animals infected with *S. duttoni*, was unable to find any immunological difference between the spirochaetes of successive relapses, and failed even to demonstrate the presence of antibodies.

The problem of immunity in spirochaetal diseases, whether dependent on residual infections, or on the development of immune bodies ("sterile immunity"), is discussed by ARISTOWSKY and WAINSTEIN (1929) who found that it was possible to produce immunity against *S. recurrentis*, by the inoculation of dead spirochaetes. Also RUSSELL (1933) immunized *C. gambianus* against a West African strain by the inoculation of dead spirochaetes combined with immune serum. On the other hand, KRANTZ (1932) produced only a comparatively transient immunity by the inoculation of living spirochaetes and immune serum, and found that the administration of immune serum at the crisis of the infection actually had a harmful effect. KROÓ (1934) found that after the inoculation of dead fowl spirochaetes into one-day old chicks no immunity developed, but adult birds similarly inoculated developed a well-marked resistance.

BELEZKI and UMANSKAJA (1929) consider that antibodies, spirochaetolysins, play the most important part in protection against spirochaetes, as evidenced by their gradual dissolution in the organs and blood. Phagocytosis, although it takes place, is said to be comparatively feeble and to play a very subordinate part in the disappearance of the organisms. This view is supported by KALAJEW (1931) ; also by KRITSCHEWSKI and RUBINSTEIN (1931), who found that both splenectomy and blockage of the reticulo-endothelial system did not abolish acquired immunity to *S. duttoni*. In addition, VELU, BALOZET and ZOTTNER (1931b) found that splenectomy, either before or after infection with *S. hispanica*, had no effect on the course of the disease. LEVADITI, MARIE and LÉPINE (1931), like KALAJEW (1931), concluded that the production of immunity is under the control of the nervous system, since it does not develop if the nervous centres are affected functionally or anatomically by a local infection. On the other hand, PLAUT and GRABOW (1930), in detailed experiments with *S. duttoni*, found that the termination of each attack was not dependent on the production of antibodies, and the sudden death of the spirochaetes is regarded as due to factors which have not yet been explained. KROÓ (1934), in the case of fowl spirochaetosis, considers that the development of spirochaeticidal antibodies and obvious immunity are different factors which vary at different periods in the life of the fowl.

The blood of certain animals seems to possess spirochaeticidal properties against *S. duttoni*, for CUBONI (1929b) found that the fresh serum of cattle, sheep and goats invariably killed all spirochaetes within 1–2 hours at 37°C. This property disappeared when the serum was inactivated by heating.

NOHIRA (1929) tested the immunity of the offspring of mice infected with a Manchurian strain. His results indicated that the offspring are often resistant, owing to the passage of immune bodies through the placenta, and not to antenatal infection of the young themselves. On the contrary, in the case of guineapigs infected with *S. hispanica*, REMLINGER and BAILLY (1929d), found that the spirochaetes passed to their offspring at all stages of gestation, but the milk was not infective.

Residual Brain Infections.

The discovery that relapsing fever spirochaetes may persist in the brains of animals for considerable periods after apparent recovery, has proved a valuable aid in the study of natural reservoirs, for many animals whose blood was negative have been found to harbour the infection in the central nervous system, and the inoculation of brain tissue is now a routine procedure in any such investigations. The factors influencing the production of such residual infections, which are by no means invariable, have been the subject of much discussion. According to KRITSCHEWSKI and BRUSSIN (1931) the different races of relapsing fever spirochaetes show varying degrees of neurotropism and somatropism, the African strains in general being highly neurotropic, and the Russian (presumably louse-borne) strains highly somatropic and very little neurotropic. This view is opposed by KOLLE, PRIGGE and ROTHERMUNDT (1931) who consider that the persistence of spirochaetes in the central nervous sytem depends essentially on low virulence and feeble development of antibodies; and ROTHERMUNDT (1928) records experiments with a Russian strain, usually not producing residual brain infections, which after its virulence had been lowered in various ways acquired the property of producing persistent brain infections in mice. HIROKI (1932), in similar experiments with a Manchurian strain of relapsing fever, found that altering the virulence did not affect the number of residual brain infections, and later (1933) in a statistical analysis of the comparative mortality of the African strains, which often produce brain infections, and the Manchurian strains, which do not possess this property, showed that there was no significant difference between them. His results are entirely opposed to those recorded by PRIGGE and ROTHERMUNDT and support the view that spirochaetes persist in the brain by virtue of a specific neurotropic character.

Alterations in the percentages of residual brain infections produced in mice by strains of *S. duttoni* and *S. hispanica* respectively, have been recorded by ROTHERMUNDT (1932). A strain of *S. duttoni* which constantly produced brain infections in 1926, four years later produced only 30 per cent. infected, but after passage through a human subject the incidence rose to 55 per cent. Similar results were obtained with the *crocidurae* strain of *S. duttoni*, and also with *S. hispanica*, but in the opinion of the reviewer, the possibility of different strains of mice varying in their susceptibility to neurotropic infection has never been taken into calculation and makes it difficult to assess the value of these and other similar experiments. SAGEL (1930) studied four African strains, two of which produced brain infections in the mouse, whilst the other two did not. A strain of *S. duttoni* and a Moroccan strain were found to lose their serological distinction, and to have increased their virulence to man, as a result of brain passage. REMLINGER and BAILLY (1929b), using a strain of *S. hispanica*, found that when inoculated intracerebrally spirochaetes persisted in the brains of guineapigs, and also in various refractory animals such as the fowl, pigeon and tortoise. In the latter, the spirochaetes remained alive approximately the same length of time as infected blood kept in glass pipettes (about 45 days).

MATHIS and DURIEUX (1930) found that residual brain infections were a constant feature in mice inoculated with the Dakar (=*crocidurae*) strain of *S. duttoni*, and might persist up to 235 days after the original inoculation, and they recommend the use of brain emulsions

for inoculation instead of blood, in order to reduce the number of animal passages necessary for the maintenance of this strain in the laboratory (MATHIS and DURIEUX, 1931). It is necessary, however, to be sure that residual brain infections are a feature of the strain, for LAGRANGE (1931), using a strain of *S. duttoni* obtained from BRUMPT, found that only one out of 11 mice showed a brain infection, although the majority of rats similarly inoculated had residual infections, the brain of one animal being infective 242 days after the original inoculation.

In the case of Californian relapsing fever, COLEMAN (1934) found that infection of the brain in mice did not persist after the blood had ceased to be infective. CUBONI (1929a) demonstrated residual brain infections in young guineapigs infected with *S. duttoni* up to 44 days after the disappearance of spirochaetes from the circulation. *S. hispanica*, which more readily infects guineapigs, has been recovered from the brains up to 100 days after inoculation (PAMPANA, 1929).

The conditions affecting the production of these residual infections have been studied by VELU, BALOZET and ZOTTNER (1931b) using guineapigs infected with *S. hispanica*. No matter what method of inoculation was used the spleen became infective as quickly as the brain, and it was concluded that the spirochaetes persisted in the brain because of the weakness of the defence mechanism in that part of the body. Splenectomy before or after infection, was found to have no effect on the course of the disease, and neither splenectomy nor blockage of the reticular-endothelial system caused spirochaetes to reappear in the circulation of animals with residual infections. SEMZOVA (1931) claims that alcohol greatly increases the chance of residual brain infections, for when mice that had been given 0·3 cc. of 15 per cent. alcohol daily for varying periods, were injected with *S. duttoni*, 37·1 per cent. of the brains contained spirochaetes, as compared with only 19·4 per cent. in the case of normal mice similarly infected.

The form in which spirochaetes persist in the brain is still the subject of discussion, LEVADITI, ANDERSON, SELBIE and SCHOEN (1929 and 1930), and also REMLINGER and BAILLY (1929b), maintaining that there is an invisible stage, whilst BEUNDERS (1932) and BEUNDERS and VAN THIEL (1932), although admitting the possibility of such an ultramicroscopic stage, incline to the view that spirochaetes, as such, persist in the brain, but are generally so rare as to escape notice.

Mixed Infections.

It is well known that when a susceptible host is simultaneously inoculated with trypanosomes and spirochaetes, the course of the infection is often prolonged.

VASSILIADIS and JADIN (1930) found that when mice containing large numbers of *S. hispanica* in their blood were inoculated with *Trypanosoma rhodesiense*, the incubation period of this latter infection was slightly prolonged and the death of the animal a little delayed. The action of *S. duttoni* on *Tr. pecaudi* was found to be much more marked, the incubation period being prolonged from 2 to 12 days. On the other hand, VELU, BALOZET and ZOTTNER (1931a) found that the simultaneous inoculation of *S. hispanica* and *Tr. marocanum* had no effect on the development of either infection.

KAWAMURA (1931) made the interesting observation that even one strain of spirochaetes might influence the action of another strain in the same host. Thus in mice inoculated with strains of *S. duttoni* and *S. hispanica*, either simultaneously, or separately with an interval of 24–48 hours, the resulting mixed infection persisted longer than either of the infections alone. Both

strains were recovered from the brains of mice after the parasites had disappeared from the circulation. The simultaneous inoculation into mice of three strains of relapsing fever, *S. recurrentis*, *S. duttoni*, and *S. marocana*, is stated by RUBINSTEIN and KAPUSTO (1931) to have resulted in the appearance of a new race of spirochaetes, serologically distinct from the three original strains. The new race was not simply a mixture, for it was passaged through mice immunized against each of the three original strains and retained its antigenic properties. It remained constant for six passages in mice, but on the seventh passage the three original strains reappeared. It is difficult to accept these remarkable observations without independent confirmation, for strains of spirochaetes, especially those producing residual brain infections, are very liable to become mixed in the laboratory, as shown by NICOLLE and ANDERSON (1929d).

KAWAMURA (1931) also studied the effect of two strains of spirochaetes, *S. duttoni* and *S. hispanica*, on mice infected with *Trypanosoma brucei*. When inoculated simultaneously the duration of life of the infected animals was about 22 days, as compared with only 4 days when the spirochaetes were inoculated 2 days after the trypanosomes. Also, in mixed infections, the removal of the spirochaetes by a dose of solganal, resulted in the mice dying of trypanosomiasis within 6 to 7 days, whilst untreated mice lived 24 to 25 days. The spirochaete is supposed to act by strengthening the natural resistance of the organism, but in mice which had been "blockaded" or splenectomized, it produced the same effects as in normal mice.

Similar results are recorded by GRILLO and KRUMEICH (1934) in the case of guineapigs infected with either *S. usbekistanica* (=*persica*) or *Spirillum minus*, and *Trypanosoma brucei*. In addition, the life of guineapigs infected with *T. brucei* was prolonged by the injection of chemical substances, which raised the body temperature, or which produced alterations in the general metabolism. These authors are of the opinion, therefore, that in mixed infections the effect of the secondary infection is to give an additional stimulus to the defence mechanism of the host.

Therapeutic Uses.

Certain authors recommend the use of relapsing fever, instead of malaria, for therapeutic purposes.

Owing to the mildness of the fever, the invariable spontaneous cure, and the ease of inoculation, MÁS DE AYALA (1931) regards *S. hispanica* as the most satisfactory agent, and gives details of a clinical study of 230 cases. Direct vein-to-vein inoculation of 2–3 cc. of blood during a febrile attack is said to give the best results. REMLINGER and BAILLY (1929c) call attention to the advantages of this strain, for it can easily be maintained in guineapigs. The virus can be transported either by means of infected ticks, or by simply defibrinating infected blood and preserving it in glass pipettes plugged with cotton wool. Such blood was found to remain virulent for at least 20 days at room temperature (REMLINGER and BAILLY, 1930a).

Cultivation.

A simple new culture medium made from egg, which does not require the addition of serum, has been described by LI YUAN-PO (1933), who maintained strains of *S. recurrentis* in this medium for at least 38 generations without the cultures losing their virulence for mice.

SCHARRER (1934) found that this medium gave good results with the fowl spirochaete, equal to any of the ordinary media containing serum. MANTEUFEL and DRESSLER (1933) also obtained excellent results with this method, but in addition describe a new medium, consisting of pieces of allantoic membrane in Tyrode's solution, in which strains of *S. hispanica* remained virulent for at least 38 passages. On the other

hand, CONSTANTINESCO (1931), using a strain of *S. duttoni*, found that when cultured in plasma containing pieces of fowl embryo, or mouse brain and spleen, the spirochaetes rapidly lost their virulence.

MARCHOUX and CHORINE (1933), using a slight modification of Galloway's method, claim to have obtained cultures of an invisible but virulent phase of the fowl spirochaete. Cultures were obtained both from the blood and organs of infected fowls and also from infected ticks. The necessary anaerobic conditions were sometimes obtained by adding *Bacillus eucarians* to the medium, and mixed cultures of this nature, continued for at least 62 passages, still remained virulent.

LAUDAUER (1931) made further simplifications in this medium and studied the factors influencing the growth of the spirochaetes. JAHNEL (1933), using fowl spirochaetes grown in a serum medium, found that their virulence fell lower and lower with successive passages, especially if the cultures were grown at 37° to 40°C. After 43 subcultures the 4-day old cultures were still virulent, but 8, 12, 13 and 16 day-old cultures were all non-pathogenic. In earlier passages the virulence could be restored, but ultimately the spirochaetes lost their pathogenicity, although the ordinary growth and motility was unaffected.

Using four strains of relapsing fever grown in a simple medium of inactivated horse or rabbit serum diluted with saline, MORODER MUEDRA (1929) also found that cultures of spirochaetes might become completely negative to microscopical examination but retain their virulence. Very often these seemingly negative cultures showed small granules, resembling micrococci, but these organisms failed to grow on agar slopes. Out of 17 mice inoculated with cultures showing no trace of spirochaetes, 15 became infected with spirochaetosis after 2 to 4 days.

EBERSON and MOSSMAN (1931), for *S. hispanica*, recommend the use of hormone broth containing brain mash, or of a sodium-citrate dextrose medium containing brain tissue. Cultures were found to remain virulent for 2 months at 38°C. A life-cycle is stated to occur in the cultures, passing from a granular stage to the adult spirochaetal form, and the preparation of a cinematograph record of this evolution is said to be in progress.

SEGUIN (1930), in cultures of *S. calligyra* from genital condylomata, observed very small spirochaetes 1 to 2 μ long, each with a drawn-out extremity resembling a flagellum. These forms appear as mere granules under dark-ground illumination. All intermediate stages were found between these minute forms and the ordinary spirochaetes.

HINDLE and ELFORD (1933) have shown that spirochaetes, including *S. pallida*, pass readily through graded collodion filters, and this method furnishes a simple and effective means of separating them from other organisms and also of estimating their diameters.

Staining Methods.

To facilitate search for spirochaetes in blood PAMPANA (1931) recommends the following solution which keeps for months :—Methylene blue (B extra) 2 gm. ; distilled water 100 cc. ; dissolve, filter, and add 4 cc. of formalin and 10 cc. of glacial acetic. The blood is obtained in thick drops, and dried rapidly by holding over the microscope lamp. The stain is poured on and allowed to act for 10 minutes, then rapidly washed in running water, blotted and dried. The spirochaetes and nuclei of the leucocytes stain blue, and stand out amid unstained surroundings. WEISS (1929) finds that mordant methods give good results, since spirochaetes have a greater affinity for acid dyes, whilst bacteria usually take basic dyes. He recommends that the material containing spirochaetes be placed

on a slide in a drop of 5 per cent. glacial acetic acid, and then put in the incubator for 15 minutes, evaporation being prevented by covering with a hollow ground slide. The drop is then spread out on the slide and allowed to dry. The slide is then covered with a mordant prepared by mixing one part of a solution of 100 gm. tannic acid in 100 cc. of 95 per cent. alcohol, with two parts of undiluted formalin containing 7·5 per cent. acetic acid. After 2–5 minutes the excess mordant is washed off with warm water, and the film stained with a mixture of acid and basic dyes, such as gentian violet and acid green; brilliant green with acid violet, or with acid fuchsin, etc. The slides are stained for 2–5 minutes in a saturated solution of the basic dye, then washed off with water and covered for 10–30 minutes with the acid dye in 30 per cent. alcohol. The slides are finally washed in water, and allowed to dry without heating.

DU (1931) recommends the use of carbol fuchsin for one minute, after dehaemoglobinizing for 5 seconds with 6 per cent. acetic acid in 95 per cent. alcohol. After trying eight different methods VAN DEN BERGHE (1931) found the following to give the best results:—

> Fix the film for 2–4 minutes in Rüge's formol acetic, or in alcohol containing 10 per cent. formalin; allow to dry, and stain for 2–3 minutes in a 3 per cent. aqueous solution of Victoria Blue 4 R (Grübler). Wash off the stain in water, and allow to dry.

General.

The necessity for the identification and control of relapsing fever spirochaetes maintained in laboratories is emphasized by NICOLLE and ANDERSON (1929d), who mention some examples of errors in the identification of well-known strains. They consider that the relapsing fever spirochaetes seem to fall into two groups: a very homogeneous *S. duttoni* group, all the strains of which are serologically identical, in marked contrast with the remaining forms, all of which show great variation and a tendency to break up into serologically different races, so that one could almost form new species out of each strain. The authors' views as to the specific identity of the strains of relapsing fever occurring in Spain and North Africa, have been criticized by DELANOË on the grounds that too large doses of spirochaetes were used in their cross-immunity tests. Accordingly, NICOLLE and ANDERSON (1932) repeated the tests with four races isolated from cases of Spanish-African relapsing fever, using different doses and methods of infection, and showed that the dosage is of no significance, and the races of spirochaetes isolated from different individuals showing the same type of disease can all be distinguished by cross-immunity tests, as well as by agglutination or lysis.

Many species of mammals have been shown to be susceptible to infection with various strains of spirochaetes. (See above "animal reservoirs"). REMLINGER and BAILLY (1929a) in the case of *S. hispanica* found that in addition to the invariable susceptibility of the guineapig, the hedgehog, wild rats and wild mice could be readily infected, also with difficulty rabbits, young dogs and in one case a 3 months' old kitten. Adult cats were refractory, also the fowl, pigeon, martin, tortoise, frog and fishes. BODECHTEL (1930) found that *S. recurrentis* and *S. anserina* might be found in the blood of lizards, frogs and goldfish inoculated with these spirochaetes, but the organisms merely persisted for a few days and there was no evidence of multiplication.

The susceptibility of spirochaetes to the action of various cytolytic agents *in vitro*, is well shown by the action of sodium ricinolate which has been found by VIOLLE (1934) to kill spirochaetes, even in dilutions

of 1 : 1,000 in media containing proteids. The exposure of *S. duttoni* to the total rays from a mercury lamp of 500 watts at 40 cms. for 18 to 30 minutes is said by LEVADITI, VAISMAN and PAÏC (1934) to destroy the reproductive capacity without affecting the motility of these organisms.

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KALAJEW, A. W. (1931), **29,** 211.
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KATZ, D. I. (1930), **28,** 298.
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KEMP, H. A., MOURSUND, W. H. & WRIGHT, H. E. (1933), **31,** 86.
— — — (1934), **31,** 508.
KLEINE, F. K. & KRAUSE, M. (1932a), **29,** 559.
— — (1932b), **30,** 11.
— — (1934), **32,** 297.
KNOWLES, R., GUPTA, B. M. D. & BASU, B. C. (1927), **27,** 110.
KOLLE, W., PRIGGE, R. & ROTHERMUNDT, M. (1931), **29,** 209.
KRANTZ, W. (1932), **30,** 365.
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— & BRUSSIN, A. M. (1931), **29,** 209.
— & DEMIDOWA, L. W. (1932), **29,** 561.

Kritschewski, I. L. & Dvolaitskaya-Barischewa, K. M. (1931), **29,** 208.
— & Rubinstein, P. L. (1931), **29,** 211.
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Lagrange, E. (1931), **29,** 560.
Landauer, E. (1931), **29,** 212.
Lasnet (1930), **28,** 297.
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Lebedewa, M. N. & Galanowa, N. W. (1932), **29,** 562.
Le Gac (1931), **29,** 206.
Legge, R. T. (1933), **30,** 721.
Levaditi, C., Anderson, T., Selbie, F. R. & Schoen, R. (1929), **27,** 695.
— — — — (1930), **28,** 302.
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— — (1934a), **31,** 505.
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— — & Advier, M. (1933), **31,** 87.
— — — (1934), **31,** 505.
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Moroder Muedra, J. (1929), **27,** 701.
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— — (1929a), **26,** 658.
— — (1929b), **27,** 108.
— — (1929c), **27,** 109.
— — (1929d), **27,** 693.
— — (1930), **28,** 731.
— — (1932), **29,** 556.
— — & Colas-Belcour, J. (1929), **27,** 107.
— — — (1929), **27,** 692.
— — & Laigret, J. (1932), **30,** 10.
— — & Le Chuiton, F. (1931), **28,** 731.
— Laigret, J. & Sicard, M. (1933), **30,** 721.
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Palmer, J. H. & Crawford, D. J. M. (1933), **30,** 721.
Pampana, E. (1929), **27,** 696.
— (1931), **28,** 737.
de Paoli, P. (1930), **28,** 298.
Pavlovskiĭ, E. N. (1932), **30,** 722.
Plaut, F. & Grabow, C. (1930), **28,** 301.
Porter, G. S., Beck, D. & Stevens, I. M. (1932), **30,** 361.

Remlinger, P. & Bailly, J. (1929a), **27,** 107.
— — (1929b), **27,** 108.
— — (1929c), **27,** 696.
— — (1929d), **27,** 700.
— — (1930a), **27,** 697.
— — (1930b), **28,** 303.
Robertson, R. C. (1932), **30,** 360.
Rosenholz, H. P. (1927), **24,** 685.
Roskin, G. & Levinson, L. B. (1930), **27,** 694.
Rothermundt, M. (1928), **27,** 111.
— (1932), **30,** 13.
— & Wichmann, F. W. (1932), **30,** 362.
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Russell, Helen (1931), **28,** 730.
— (1932), **30,** 362.
— (1933), **30,** 363.

Sachs, Albert (1934), **32,** 296.
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Scharrer, B. (1934), **31,** 853.
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— Manceaux, A. & Balliste, R. (1933), **31,** 87.
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— Kotrba, J. & Fischl, V. (1934), **31,** 851.
Sternberg, E. J. & Pines, A. I. (1933), **30,** 723.
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Todd, J. (1930), **27,** 694.
Toyoda, H. (1931), **28,** 738.
Tschirejkin, W. Ch. (1930), **27,** 695.

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Vassiliadis, P. & Jadin, J. (1930), **28,** 306.
Velu, H., Balozet, L. & Zottner, G. (1931a), **28,** 734.
— — — (1931b), **28,** 736.
Violle, H. (1934), **31,** 852.

Weiss, E. (1929), **27,** 115.
Weller, B. & Graham, G. M. (1930), **28,** 299.

Yuan-Po, Li (1933), **30,** 366.

LEPROSY.

LEPROSY REVIEW. 1934. Oct. Vol. 5. No. 4. pp. 149–197. With 15 figs. on 4 plates & 1 text fig. Quarterly Publication of the British Empire Leprosy Relief Association, 131 Baker Street, London, W.1. [2s.]

The most important paper in this issue is by F. G. ROSE on the curability of leprosy, based on many years work in charge of a campaign against the disease on modern lines in British Guiana, with the aid of a leper hospital and dispensaries for out-patient treatment of early cases found by surveys. Still more important, he has been able to follow up for long periods nearly all the discharged patients in a manner not yet possible in other leprous countries, and to prove that not more than 14·1 per cent. have shown lasting relapses. Tables of the data are given and Dr. ROSE's own summary as follows speaks for itself.

"1. Of 801 patients suffering from leprosy under observation in British Guiana from 1926 to 1934, 138 have died, and 16 have left the country.

"2. Of the remaining 647, 180 are cases spontaneously arrested, leaving 467 who have undergone active treatment during this period.

"3. Eighty-six of these received treatment for less than a year, leaving 381 whose ultimate fate is considered.

"4. Two hundred and fifty-seven were early cases, of whom 76 are now arrested, 66 quiescent, and 66 improved.

"5. One hundred and twenty-four were advanced cases, of whom 22 are arrested, 15 quiescent, and 66 improved.

"6. It is suggested that arrested and quiescent cases in whom function has been completely restored should be termed recovered in addition.

"7. Of the 142 early quiescent and arrested cases, 100, and of the 37 advanced cases 10, have completely recovered.

"8. Ninety-eight cases have become arrested, of whom 13 have eluded observation.

"9. Of these 85, 14·1 per cent. have relapsed and have not yet become re-arrested.

"10. Relapse generally occurs within the first two years after the arrested stage has been reached.

"11. Treatment should be continued for at least six years after arrest.

"12. An arrested case may be deemed cured after six consecutive years of inactivity.

"13. Special attention should be devoted to childhood infection."

The Editor of the review has adopted the unusual procedure of asking leprosy experts, working under totally different conditions in far distant countries, to criticize Dr. ROSE's paper. The most experienced of these is Dr. E. MUIR of Calcutta, who strongly supports Dr. ROSE in the following findings among others.

"(2) The table of results in early cases corresponds to the results that we obtain in India in places where the patients attend regularly and are efficiently treated. (3) The results in advanced cases also correspond closely with results obtained in India under favourable circumstances." [The reviewer declined an invitation to comment on Dr. ROSE's paper as he accepts unreservedly the records and conclusions of that able and experienced worker.]

The evaluation of the results of treatment in incipient leprosy is once more dealt with by J. RODRIGUEZ, who repeats his experience that chaulmoogra preparations are less effective in the very early and often bacteria-free stages than in those with more developed dermal lesions containing abundant lepra bacilli. [This may possibly be due to the fact that destruction of the bacilli leading to a gradual production of immunity is an important factor in recovery.]

In a further yearly review of his leprosy work in Korea R. M. WILSON records the value of localized anaesthesia and nerve thickening in early diagnosis. In treatment he still finds injections of 5 to 7 cc. doses of pure freshly obtained hydnocarpus oil the cheapest, most satisfactory and most painless method. In 70 to 80 per cent. of early cases marked improvement is obtained. He has noticed a pellagroid condition of cases in the spring, and that most of the Korea cases come from the southern humid half of the country. A paper on dye treatment is dealt with below under that heading. *L. Rogers.*

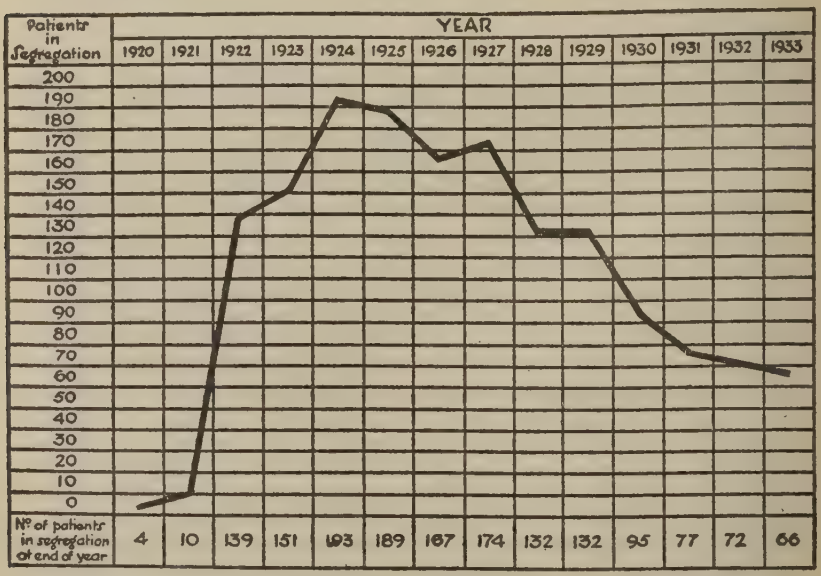
MARCHOUX (E.). La lutte contre la lèpre dans les colonies françaises. [**Anti-Leprosy Measures in the French Colonies.**]—*Internat. Jl. Leprosy.* Manila. 1934. Aug.-Oct. Vol. 2. No. 3. pp. 311–314.

"The antileprosy campaign that has long been carried on in the French colonies, based on the internment of cases, has not given the expected results. The conditions of the measures in force, and a widespread idea that the disease is hereditary, have resulted in an attitude strongly antagonistic to the system in spite of efforts to ameliorate the circumstances of those affected, and the disease has not only persisted but is continually spreading. To deal with the situation the Minister of Colonies has appointed a permanent commission, composed of Drs. Marchoux, Jeanselme, Gougerot and Burnet, which has considered a revision of measures for leprosy control for the colonies.

"The principles approved are, briefly, that the disease is a communicable one, the germ of which escapes from the tissues only when there are ulcers, in the absence of which even prolonged contact is not dangerous; ulcers of the mucosa present a greater difficulty than those of the skin, and ulcers may be precocious and inconstant. Diagnosis should, therefore, be made as early as possible; this requires a skilled specialist, but an educated populace will make diagnoses by themselves, so there is need of instruction of the public in regard to this, and also regarding the dangers of contagion and the value of early treatment. The first task is to enumerate the cases and to classify them—cutaneo-mucous, nervous and latent being the three types enumerated. Methods of providing treatment under different conditions, the desiderata as regards hospitalization, and the practicable methods of educational propaganda are enumerated." *L. R.*

GRANT (Alan M. B.). **Leprosy at Nauru since 1928.**—*Internat. Jl. Leprosy.* Manila. 1934. Aug.–Oct. Vol. 2. No. 3. pp. 305–310. With 1 text fig.

This important article reports the further progress of the effort to reduce the high incidence of leprosy at Nauru by modern methods since the publication of Dr. BRAY's article in 1930 [see this *Bulletin*, Vol. 27, p. 997], which is conveniently reprinted in this issue of the journal. The 2,500 inhabitants are examined frequently for early cases of leprosy and only the infectious ones are segregated in accordance with the advice of L. ROGERS. The accompanying chart and table show that the infectious bacteriologically-positive cases have fallen from 193 in 1924 to 66 at the end of 1933, or by almost two-thirds within one decade, but the rate of decline is now less, partly owing to the admission of 16 cases without bacteriological examination in 1932. In addition 155 uninfectious cases are attending clinics as out-patients, and none of these are discharged as apparently cured until after five years treatment with two years' freedom from all active symptoms ; they live in separate houses and apart from any children. Hydnocarpus esters are used in treatment. In the last five years 83 have been paroled, and only 22 have relapsed, but 21 of 48 of discharged clinical cases were again given treatment for relapse. Nine have died in five years. Infants born in the isolation station are separated from their parents at birth. The author concludes that leprosy is slowly decreasing, that early diagnosis by frequent inspections has been of paramount importance, and that "the division of the cases into infectious and uninfectious and the different treatment of these groups has given better results than would have resulted with compulsory segregation of every case of leprosy."



Graph showing the total number of cases of leprosy in segregation at the end of each year since the appearance of the disease at Nauru.

[Reproduced from the *International Journal of Leprosy.*]

Admissions, discharges and relapses at both clinic and isolation station, Nauru, during the five years, 1929–1933, inclusive.

Occurrence.	Year.				
	1929	1930	1931	1932	1933
Admissions, new patients to station ...	5	4	4	16*a*	4
,, ,, ,, clinic ...	29	60	31	43	8
Discharges, patients from station	4	39	19	21	—
,, ,, ,, clinic	6	2	3	22	15
Relapses, admitted to station	—	—	—	—	11*b*
,, ,, ,, clinic	—	—	—	—	21*b*
Total number of cases in station	132	95	77	72	66

a This number was admitted without bacteriological examination.
b Total for the five-year period. *L. R.*

GOMES (J. M.). A lepra no Estado de S. Paulo. (Notas endemiologicas.) [**Leprosy in the State of S. Paulo.**]—*Mem. Inst. Oswaldo Cruz.* 1934. July. Vol. 28. No. 3. pp. 317–387. With 7 plates (2 maps). English summary pp. 388–390.

Leprosy has increased gradually in S. Paulo State during the last century with more active foci in certain areas requiring most attention. The great majority of these are found on non-sandy soil with high humidity. Examination of lymphatic glands and allergic manifestations indicate that a large number of contacts with open lepers become infected without showing signs of the disease, mostly during childhood, and more from their mothers than from their fathers. *L. R.*

DENNEY (O. E.). **The National Leprosarium, Carville, La. Review of the More Important Activities during the Fiscal Year ended June 30, 1934.**—*Public Health Rep.* 1934. Nov. 16. Vol. 49. No. 46. pp. 1359–1365.

In spite of a reduced budget the activities have been maintained at this institution. New admissions numbered 64, and 20 were paroled, leaving 361. Chaulmoogra oil orally in from 3 to 155 drops three times a day was used in 225 cases, and benzocaine-chaulmoogra oil intramuscularly twice weekly in 123, while 50 were on esters intramuscularly. Several dyes were tried "without encouraging results." The usual special services were maintained. Initial apparent increase in acid-fast bacilli from leprous lesions was obtained on a variety of culture media, possibly due merely to concentration through autolysis of the tissues inoculated with them, but no active subcultures could be obtained. *L. R.*

SHARP (Leonard). **Further Report on Bunyoni Leper Colony, Kigezi, for 1933–34 with Statistics.**—*East African Med. Jl.* 1934. Nov. Vol. 11. No. 8. pp. 245–255.

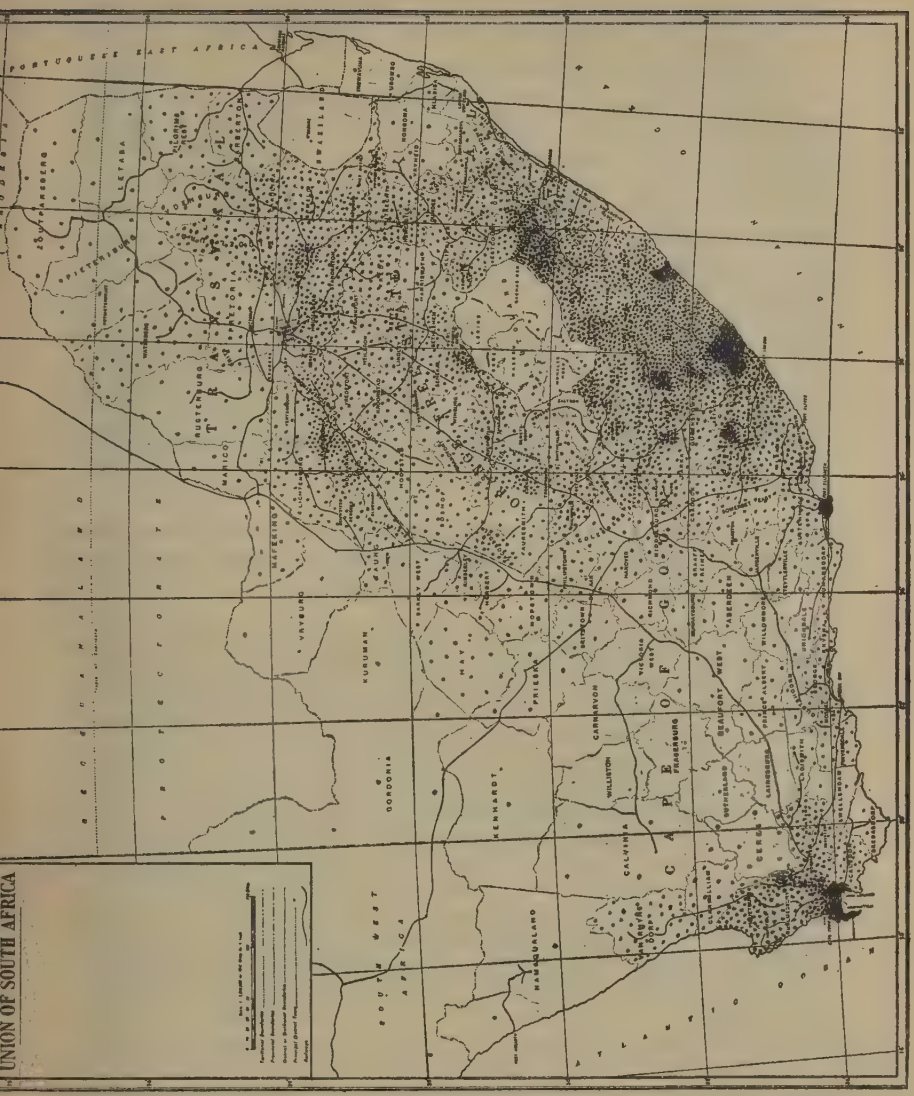
During a further year's work at this West Uganda island settlement the numbers under care increased from 300 to 522. Improved buildings and additional land for cultivation have been supplied and plantations of eucalyptus trees made. Over 100 children are being taught and 33

have shown arrest of the disease. A much needed home for untainted children has been built, for 57 per cent. of the children of lepers have become infected, and without separation from their parents probably 75 per cent. would eventually develop the disease. One-third of all the lepers in the colony are children under 15 years of age, 75 per cent. of whom are early or mild cases, and among 194 early nerve or cutaneous cases 43 per cent. have become arrested during the year and they constitute 92 per cent. of the total arrested cases. Similar tables to those of the previous year's report are given with 46 per cent. improved in addition to the 23 per cent. arrested, including all treated for three months or over. They are subdivided into numerous tables, three with only 2 to 5 cases and nine more with less than 20 cases, but the general conclusion come to is that those with no drug treatment did best and those with most injections did worst, brilliant green being better than chaulmoogra oil preparations. In view of the shortness of the treatment in some at least the results of the five years' treatment, found by Dr. Rose to be necessary to obtain the best results with chaulmoogra preparations, will be awaited with interest. *L. R.*

Union of South Africa. Annual Report of the Department of Public Health Year ended 30th June, 1934. [**Leprosy** pp. 31–45. With 6 maps (1 folding).]

This report is by Sir E. N. Thornton and it takes a more hopeful view than earlier ones. The expenditure has fallen from £204,000 fourteen years ago to £154,000 when the health department took over the work in April 1924 and to £97,428 in the last financial year, which is still nearly one-fourth of the cost of the whole public health work. After some historical data and information for the enlightenment of the public on the low infectivity and chronicity of the disease, it is stated that : " In its early stages the disease is most amenable to treatment. Chaulmoogra oil still holds the field." The disease is decreasing among Europeans and there are probably fewer cases than once thought among non-Europeans. Tables show 2,155 total, but only 95 pure Europeans, segregated in leper institutions, which have now all been converted into well staffed hospitals. In addition there are 1 certified and 6 home segregated Europeans, 1,542 in all discharged probationally, but still under surveillance, and 889 released from surveillance as no longer requiring to be watched. Treatment remains voluntary and the ethyl esters and sodium salts of the lower melting point fatty acids are the most popular preparations. Compulsory segregation is still considered the only sound method in S. Africa.

An instructive study of leprosy incidence among non-Europeans from 1900 to 1930 has been made by Dr. J. F. Wood and is illustrated by maps. The data show a rate of 1·9 per mille, 855 cases, in the Orange Free State, mostly the Northern and Middle districts. From the Transvaal there were 2,860 admissions, or 2·4 per mille, with most on the border of the Vaal River. Natal showed 1,424 cases, or 1·2 per mille, mostly along the Basutoland border. The Cape Province had 2,488 cases, or 1·5 per mille. The four smaller native areas show rates of from 2·6 to 3·7 per mille. A spot map of all the cases shows a remarkable concentration of cases in the eastern areas, especially around the hilly Basutoland, and in a small one near Cape Town. [The distribution of high rainfall is similar.] *L. R.*



Relative incidence of leprosy in non-European population of South Africa during the period 1900–1930.

[Reproduced from *Union of S. Africa Annual Report of the Department of Public Health for Year ended 30th June, 1934.*]

Yu (K. Y.). **Leprosy among Natives of Manchuria.**—*Jl. Oriental Med.* 1934. Nov. Vol. 21. No. 5. pp. 67–71.

This is a brief account of four cases of leprosy among the indigenous inhabitants of Manchuria, who were probably infected during long residence in Mukden villages of Shantung mining and artisan immigrants. *L.R.*

GALT (Curtis M.) & YAWT (Noi). **Kiulungkiang Married Lepers' Settlement.**—*Internat. Jl. Leprosy.* Manila. 1934. Aug.–Oct. Vol. 2. No. 3. pp. 315–317.

On account of too many lepers leaving this colony in China early in 1932 married lepers were allowed to start a neighbouring village of their own on a self-supporting basis, and lepers in the colony were permitted to marry and move to the village, treatment being supplied to them as before. Only those in fairly good health, who could build a modest house and cultivate a garden, were granted such leave. It now includes forty-seven couples, together with six children taken to the village and twenty-five born there, of whom seven have been adopted by friends and 18 await adoption, which is common custom there, and it is also hoped to provide an untainted home for children. There has been only one request for return to the parent home and no financial aid has been needed, but attendance for treatment has not been very regular. The finances of the parent colony have benefited greatly, and losses of patients have been much reduced, so the experiment is being continued with an open mind as to the ultimate results. *L. R.*

HAYASHI (Fumio). **The Anti-Leprosy Works in Various Parts of the World as I have seen them.**—*Jl. Public Health Assoc. Japan.* 1934. Sept. Vol. 10. No. 9. pp. 1–13.

This is an interesting but diffuse account of the impressions of the author during a year's tour of a number of leprous countries, in which he bears witness to good work being done. He emphasizes the number of infections of children under leper segregation, including 38 per cent. of those born at Culion, and the importance of homes for untainted children removed as early as possible from leper parents, and elsewhere in India. He prefers the Japan solution of preventing conception by doing vasectomy on the husband before marriage is allowed. He is not in favour of the Culion parole system unless the patients are followed up, which has not yet proved feasible. The surveys in India are commended. The incidence, so common in Japan, of alopecia in leprosy is discussed and the conclusion come to that a cold climate favours its occurrence. The great decline in the mortality rates among lepers in various countries is pointed out. The value of the Mitsuda reaction is stressed. With regard to treatment he says: "The efficacy of chaulmoogra oil is an obvious fact, still there are some who doubt its value." *L. R.*

VELASCO (Felix). **Frequency of Leprosy among Parents and Children: its Bearing in the Transmission and Epidemiology of the Disease.**—Reprinted from *Rev. Filipina de Med. y Farmacia.* 1934. Sept. Vol. 25. No. 9. pp. 423–433. [18 refs.]

This inquiry was carried out in the Manila leper hospital among 27 adults with 125 children of whom 80 were examined; 61, or 76·3 per cent., were leprous, 22.9 per cent. being bacteriologically positive and 77.1 per cent. clinical cases. The incidence in the children was highest where one or both of the parents were positive, lower when the father is a clinical leper and least when both parents were healthy. A noteworthy feature was that the early recognizable lesions are usually found on the bare skin surfaces which during infancy are

most frequently in contact with the skin of the mother, and these are also the frequent sites in adults. "This fact seems to justify the assumption that transmission is a direct skin to skin contact between the leper and the susceptible infant or young child." Moreover, the results of leprolin tests indicate that infection most frequently takes place in infancy. *L. R.*

WAYSON (N. E.). **Leprosy with Tuberculosis in Hawaii.**—*Public Health Rep.* 1934. Oct. 12. Vol. 49. No. 41. pp. 1201–1212.

The author discusses the well-known frequency of the association of tuberculous and leprosy infections as seen in Hawaii, where DOOLITTLE obtained 75 per cent. of positive tuberculin reactions among 1,500 school children. In a group of leprous patients tuberculin reactions were about half to one-third as frequent as in other groups. The average annual death rate from tuberculosis among 155 lepers was nearly 2,000 per 100,000, against 100 in the general population. Febrile and local leprous reactions are more common in patients with tuberculous complications, namely 70 per cent. against 15 per cent. in those without this complication. The explanation of these data is not very clear except that any complicating disease tends to increase the severity of leprosy. Methods for the control and treatment of tuberculosis may have a good effect on leprosy incidence. *L. R.*

BASU (N. K.). **Deficiency of Vitamin-B_2 (G) as an Etiologic Factor in Leprosy.**—*Ztschr. f. Vitaminf.* Berne. 1934. July. Vol. 3. No. 3. pp. 194–195.

In this short note the author states that the occurrence of pellagra in lepers led him to investigate their diet in Calcutta, where it is very poor in protein and vitamin B, especially in B_2. On giving a teaspoon daily of a preparation adjusted to the strength of marmite he found after a month improvement in the sensation of nerve forms of leprosy, but no effect in nodular cases. *L. R.*

CAMPOS (Nelson de Sousa). A prova da histamina no diagnostico da lepra maculo-anesthesica. [**The Histamine Test in Diagnosis of Maculo-Anaesthetic Leprosy.**]—*Brasil-Medico.* 1934. Dec. 29. Vol. 48. No. 52. pp. 1083–1088.

The author describes the effect of the phosphate or hydrochloride of histamine, when a needle is inserted through a drop of it into the skin not deeply enough to draw blood, or when a solution of it is injected intradermally. For the test the author uses a 1 per cent. solution or 0·1 cc. of it for injection. The procedure has already been described by RODRIGUEZ and PLANTILLA [see this *Bulletin*, Vol. 29, p. 268], but these authors used a 1 in 1,000 solution and stated that reactions are not constant if stronger solutions are used.

The effect on the normal skin and on skins showing conditions of dermatitis not associated with nerve lesions is a reddening of 3–4 mm. diameter round the site of the prick in 20 seconds, followed by a wider flush in 15–30 seconds and a wheal in 5 minutes or less. In the case of leprotic maculae this reaction fails to appear and the author regards the test as one of considerable diagnostic value in early cases where bacteriological examination has proved negative. There are certain

drawbacks or limitations to its usefulness, however, for " interpretation of results is impeded in dark skins, in lesions associated with much erythema, and in dark cicatricial spots." *H. H. S.*

LOWE (John). **A Further Note on Nerve Abscess in Leprosy.**—*Internat. Jl. Leprosy.* Manila. 1934. Aug.–Oct. Vol. 2. No. 3. pp. 301–304. With 6 figs. on 2 plates.

This brief note is illustrated by excellent photos of the external appearances in five cases, and of a large abscess of the ulnar nerve dissected out at an operation. In Dichpali the incidence among about 5,000 lepers was 2 per cent., but half of these followed reactions induced by potassium iodide. They were met with in all the nerves most commonly affected by the disease and produced evident swellings under the skin in superficial nerves. The lepra bacillus was found in half those operated on, and in nearly every case in the neighbouring thickened portion of the nerve, together with caseous-like material. Such abscesses may occasionally perforate the skin and continue discharging for years, but they may also undergo resolution, so operations are not always required unless pressure symptoms develop; drainage is unnecessary after dissecting out the abscess material when it has escaped from the nerve sheath, as not infrequently occurs. These cases are equally common in Calcutta, but more frequently involve the subcutaneous nerves. *L. R.*

WADE (H. W.). **Tuberculoid Changes in Leprosy. II. Lepra Reaction in Tuberculoid Leprosy. III. The Pathology of a Nerve Abscess.**—*Internat. Jl. Leprosy.* Manila. 1934. Aug.–Oct. Vol. 2. No. 3. pp. 279–292. With 20 figs. on 5 plates; 293–300. With 12 figs. on 2 plates.

The first of these papers discusses six cases of probable lepra reaction seen in South Africa, four diagnosed as such clinically, of which two were severe, and two showing doubtful reactions, but were bacteriologically positive. One severe reaction followed the administration of potassium iodide and was of long duration. Histologically the lesions showed only tuberculoid changes with the production of epitheloid and some giant cells, together with relatively numerous bacilli for this type, but not in the form of globi, and mostly in the advancing border in the reacting cases. The prognosis of this development is thought to be unfavourable. Excellent photos accompany the paper.

III deals with the microscopical changes in nerve abscess tissue supplied by Dr. LOWE, at Dichpali [above]. This was found to present a highly organized tuberculoid granuloma, surrounded by an inner capsule of vascular lymphoid follicles, and an outer one apparently derived from the perineurium, the whole considered to be the product of a lepra reaction in a nerve and containing few or no bacilli. *L. R.*

PESCHKOWSKY (G. W.). Steigerung der fagozitären Aktivität der polynuklearen Leucocyten als Resultat der entzündlichen Exazerbation, und das weisse Blutbild als Ausdruck des Typus der entzündlichen Reaktion bei Lepra. [**The Blood Picture in Leprosy.**]—*Internat. Jl. Leprosy.* Manila. 1934. Apr.–July. Vol. 2. No. 2. pp. 129–138. English summary.

The author describes the blood picture in leprosy as the result of an inflammatory reaction of the reticulo-endothelial system. The presence

of monocytosis is unfavourable as it indicates the dissemination of the disease with the formation of fresh granulomata with proliferative chronic inflammation. On the other hand, lymphocytosis is favourable in most cases, as it coincides with a period of convalescence and decrease of the inflammatory process. Polymorphonuclear leucocytosis accompanies an exacerbation of the disease with suppurative inflammation and subsequent destruction of the bacilli in the polynuclear leucocytes.

L. R.

LAI (Daniel G.). **The Dextrose Tolerance Test in Leprosy.**—*Amer. Jl. Trop. Med.* 1934. Nov. Vol. 14. No. 6. pp. 575–584. With 1 fig.

Sixty lepers and one normal control have been tested with the following results. The mean basic metabolic rate was 62·7. The fasting blood sugar rate varied from 62 to 124 mgm. per 100 cc., and averaged 88·5 mgm. The composite blood sugar curve was considered normal. During the test 58 per cent. showed glycosuria once or more. In 27 per cent. high, and in 19 per cent. flat, curves were seen, and the renal threshold was usually low. Thus, in spite of individual variations, uncomplicated leprosy tends to give a normal blood sugar curve, and glycosuria is apparently due to a low renal threshold commonly occurring in leprosy. *L. R.*

SARDJITO & SITANALA (J. B.). **Additional Notes on Lepra Bacilli in the Thick Blood Drop taken from Normal-appearing Skin Areas of Lepers.**—*Meded. Dienst d. Volksgezondheid in Nederl.-Indië.* 1934. Vol. 23. No. 4. pp. 159–167. [11 refs.]

These workers conclude from their investigation that the acid-fast bacilli found in drops of blood obtained from normal looking skin of leprosy patients are derived from the circulating blood, and only to a negligible degree from the tissue fluids. This indicates that the disease is disseminated through the blood stream, but the bacilli may accumulate in the capillaries and later make their way into the surrounding tissues to start new lesions. The examination of blister fluids for bacilli is therefore not advised. They found the bacilli in thick blood preparations made by pricking the healthy skin of the ear or a finger in 85 per cent. of nodular and in 17 per cent. of nerve cases.

L. R.

MOUTOUSSIS (Konstantin). Ueber die Bazillämie bei Lepra und sonstige Befunde im Blute bei Leprakranken. Vorläufige Mitteilung. [**Bacillaemia in Leprosy.**]—*Arch. f. Schiffs- u. Trop.-Hyg.* 1934. Nov. Vol. 38. No. 11. pp. 487–494. With 5 figs.

The author records the examination of the blood of 79 lepers for acid-fast bacilli with positive results in 45 of 46 nodular cases, in 13 out of 15 with macular lesions, in 6 of 9 nerve cases, and in 8 of 9 mixed ones.

L. R.

OTA (Masao) & SATO (Saburo). **Cultivation of Leprosy Bacilli and of the Tubercle Bacillus from Leprosy Tissues.**—*Internat. Jl. Leprosy.* Manila. 1934. Apr.–July. Vol. 2. No. 2. pp. 175–192. With 12 figs. on 1 plate. [30 refs.]

After giving a short account of previous Japanese work on the subject the authors describe their own attempts to cultivate the leprosy bacillus, and lay due stress on the difficulties in deciding whether acid-fast bacilli so obtained are true lepra ones. Their work is well summarized in their own conclusions as follows :—

"1. An acid-fast bacillus cultivated from leprous materials is not necessarily the leprosy bacillus. We have obtained cultures of the human tubercle bacillus from a clinically typical leprous nodule and a typical leprotic lymphoma.

"2. It is very difficult to obtain the tubercle bacillus from the blood by Löwenstein's method, even with lepers having complicating tuberculosis. We used this method in 83 cases of advanced nodular leprosy, one half with pulmonary tuberculosis, and obtained no culture of the tubercle bacillus. On the other hand we cultivated twelve strains of acid-fast bacilli which were not tubercle bacilli.

"3. In one instance, four months after an acid-fast bacillus was obtained from a leper by the blood culture method, the same organism was also recovered from a nodule removed from the same patient, demonstrating that the cultures were not contaminations from outside but that the patient had a general infection with this organism. This is important evidence that the organism is the leprosy bacillus.

"4. Two other strains of the acid-fast bacillus were cultivated from nodules from two patients. It is more difficult to obtain these organisms from the nodule than from the blood, though we believe that more positive results may be obtained by improving the technic.

"5. From the results of animal inoculations and complement-fixation and skin reactions not described in this paper it seems highly possible that the strains obtained by us are *Mycobacterium leprae.*

"6. These strains may be divided into two types according to the color of the cultures, one being whitish, the other ochre or orange-colored, though these characteristics are not always constant and in some cases whitish strains change to ochre color during subcultivation. These types we call *Myco. leprae* var. *album,* and *Myco. leprae* var. *aurantiacum,* respectively." *L. R.*

DENNEY (O. E.). **A Microscopic Study of** *Mycobacterium leprae.*—*Internat. Jl. Leprosy.* Manila. 1934. Aug.–Oct. Vol. 2. No. 3. pp. 275–278. With 25 figs. on 3 plates.

This is a brief description of the morphology of the lepra bacillus in unstained material, and is illustrated by high magnification drawings. The beading and branching of the organisms is well brought out, and also the appearances of globi masses of bacilli, which are described as disk-like colonies of organisms surrounded by a cell membrane. The significance of the granules is still unknown. *L. R.*

STEIN (A. A.) & STEPERIN (M. I.). Die spezifische Allergie bei Leproesen. [**Specific Allergy in Leprosy.**]—*Antonie van Leeuwenhoek Nederl. Tijdschr. v. Hyg., Microbiol. en Serol.* 1934. Vol. 1. No. 3. pp. 209–218. [11 refs.]

The author reports that the injection of emulsions of leprosy nodules (leprolin) into the skin produces specific reactions. In clinical cases the

appearances of the reactions are similar to those of tuberculin. In more advanced cases the reaction varies from negative results in mixed and nodular cases to positive ones in nerve cases. Healthy persons give positive reactions. Negative results are met with in persons who have had no contacts with lepers and positive ones in those who have had such contact. *L. R.*

NAKAMURA (Keiso) & KOBASHI (Shigeho). Inokulationsversuche der Menschenlepra auf Hausratten. I. Mitteilung. [**Inoculation of Leprosy to Rats.**]—*Keijo Jl. Med.* 1934. Sept. 30. Vol. 5. No. 3. pp. 184–189. With 8 figs. (1 coloured) on 2 plates.

The authors report that by the inoculation into young rats of human leprosy material after damaging the nasal mucous membrane by acid an infection will result, and also by intratesticular inoculation after removal of the thyroid gland. *L. R.*

JORDAN (Paul). Notas preliminares sobre o " gambá " como animal de experiência para a lepra. Pesquisa dos bacilos ácido-resistentes nos animais sãos. [**Opossum as Experimental Animal in Leprosy.**]—Reprinted from *Folia Clin. et Biol.* São Paulo. 1934. No. 3. pp. 85–88. German summary.

BOYÉ in Cayenne found an opossum with mutilated toes as in leprosy, and the inguinal glands and an infiltration of the bones showed acid-fast rods. An opportunity occurred to dissect opossums but no signs of leprosy bacilli were found in the nasal mucus, glands, brain or spinal cord, or in internal organs. Fifty fleas caught on the animals were also negative. Opossums were inoculated with human leprous material with negative results. *L. R.*

JORDAN (Paul). Estudo sobre o sôro leproso. Experiencias com estractos de actinomicetos como antigenos na reacção do desvio do complemento. [**Complement Reactions in Leprosy.**]—Reprinted from *Folia Clin. et. Biol.* Sao Paulo. 1934. No. 3. pp. 81–84. [11 refs.] German summary.

The author records that lepers negative to the Wassermann reaction may yet give positive results with an alcoholic extract of actinomycetes. *L. R.*

DES ESSARTS (J. Quérangal) & LEFROU (G.). Note sur le diagnostic différentiel entre les nodules élémentaires lépreux et tuberculeux dans les lésions cutanées. [**Differentiation of Skin Lesions of Leprosy and Tubercle.**]—*Bull. Soc. Path. Exot.* 1934. Oct. 10. Vol. 27. No. 8. pp. 706–709.

This is a short note on the microscopical differences between tuberculous and leprous lesions of the skin. The main points are that in leprosy the nodules stain more uniformly, but are more irregular in shape, affect the epidermis less with more irregular arrangement of the cells, are more vascular, contain few lymphocytes and giant cells, do not caseate and contain much larger numbers of acid-fast bacilli.

L. R.

GILLIER (R.). Formol-gélification des sérums lépreux. [**Formol-Gel Test with Leprous Sera.**]—*Bull. Soc. Path. Exot.* 1934. Oct. 10. Vol. 27. No. 8. pp. 709–713. With 2 figs.

The author has applied this test in 18 lepers, 2 children living with leper parents and 30 syphilitics, and concludes that the formol-gel test is positive in Wassermann negative lepers. The optimum strength is one to two drops of formol added to 1 cc. of serum. The reaction is more rapid than with syphilitic sera. *L. R.*

MONTESTRUC (E.). Lèpre et séro-floculation de Vernes à la résorcine. [**Vernes Resorcin Sero-Flocculation in Leprosy.**]—*Bull. Soc. Path. Exot.* 1934. Oct. 10. Vol. 27. No. 8. pp. 713–715.

This reaction consists in a flocculation of serum on the addition of 1·25 per cent. solution of resorcine due to an excess of euglobulins and pseudoglobulins ; it was first used in tuberculosis. The present paper records tests in 17 nerve and 34 nodular lepers, and the conclusions are come to that it occurs only in the nodular form, and that it is of no use in the diagnosis of early cases since it only takes place in those easily recognized either clinically or bacteriologically. *L. R.*

RUBINO (Miguel C.). Les antigènes lipoidiques d'organes dans le séro-diagnostic. Nouvel antigène de séro-floculation dans la lèpre. [**Lipoid Antigens of Organs in the Serodiagnosis of Leprosy.**] —*C. R. Soc. Biol.* 1934. Vol. 117. No. 35. pp. 894–897.

This is a preliminary report on the employment of lipoid alcoholic extracts of the organs of rabbits, with formalized red corpuscles of sheep as an antigen in the sero-flocculation of the serums of lepers. Extracts of the kidneys gave no reactions and those of nervous tissues slight ones, but the liver preparations were the most active. Reactions may be obtained with the sera of lepers which are negative, with those of syphilitics and other diseases. The diagnostic value of this test has not yet been worked out and purer lipoids are required than have yet been obtained. *L. R.*

PALDROCK (A.) & POOMAN (A.). Die Trypanblauquaddelreaktion bei Leprösen. [**The Trypanblue Wheal Reaction in Leprosy.**]—*Internat. Jl. Leprosy.* Manila. 1934. Aug.–Oct. Vol. 2. No. 3. pp. 271–274.

This reaction is carried out by the intradermal injection of a 1 in 5,000 watery solution of trypanblue into an 8–10 mm. patch of skin in the inner side of the upper arm and also on the intrascapular portion of the back. After twenty-four hours blue staining of the whole infiltrated area is noted on the back and a ring-like staining on the upper arm, and subsequently varying degrees of diffusion and absorption of the stain are noted. Absorption is normal on both the arm and the back in the case of maculo-anaesthetic and slight cutaneous cases in 87·5 and 100 per cent. respectively, but was accentuated in 100 per cent. of advanced cutaneous cases, the total number tested being 43 lepers. Diffusion, on the other hand was normal and unaccentuated in 93·7, 100 and 92·3 per cent. of the three types in the same order as above. *L. R.*

MUIR (E.). **The Leprolin Test.**—*Calcutta Med. Jl.* 1934. Nov. Vol. 29. No. 5. pp. 225–226. With 5 figs. on 1 plate.

This brief note records that Hansen's leprolin has been used with advantage for intradermal injections of suspensions of 1–10 to 1–30 into leprosy lesions in patients showing acquired immunity or enhanced resistance to the disease. One infiltration may cause the disappearance of well-marked lesions and 50 per cent. of all cases are suitable; treatment is much shorter than with hydnocarpus preparations. *L. R.*

DUBOIS (A.) & DEGOTTE (J.). La réaction de Mitsuda dans la lèpre. [**Mitsuda's Reaction in Leprosy.**]—*Bull. Soc. Path. Exot.* 1934. Nov. 14. Vol. 27. No. 9. pp. 802–805.

In the Mitsuda test a suspension of broken up lepromata sterilized by boiling is injected intradermally, while Bargehr's modification is a cuti-reaction done by applying the material to an abraded portion of the skin; French observers, however, have found the latter less reliable than Mitsuda's original method, which is reported on in this note, with the use of one-tenth to one-twentieth of a cc. of a phenolized solution of lepromas in saline. A positive reaction consists of a persistent infiltration of the skin of from 1 to 10 mm. in diameter and it should be noted after one, two and three weeks. Negative results occur in active cutaneous leprosy containing numerous lepra bacilli, but both maculo-nervous cases with few or no bacilli and healthy persons give positive reactions owing to their tissues not having become accustomed to the toxic material. Tests in 171 lepers and 12 healthy subjects dealt with in this paper confirm these results. *L. R.*

GOMEZ (J. M.). **The Gomez Complement-Fixation Reaction in Leprosy.** —*Internat. Jl. Leprosy.* Manila. 1934. Aug.–Oct. Vol. 2. No. 3. pp. 265–269.

Working in Brazil since 1926 the author has used glycerin broth cultures of Deycke's streptothrix incubated for 20 days at 37°C., after removal of the waxy-fatty coating by treating with olive oil and acetone until not more than 10 acid-fast bacilli are found in each microscopic field. The resulting fine whitish powder is kept in a sterile flask, and a 9 per cent. emulsion in normal saline is made and heated to 100°C. for five minutes to destroy anti-complementary reaction. 0·5 cc. of the antigen is added to 0·05, 0·1, 0·2 and 0·5 cc. respectively in four tubes and sensitized red cells added. Over 2,000 tests have been performed, including 559 lepers, 713 suspicious cases and 154 other cases. In the typical cases nodular and mixed ones gave 96·7 and 95·4 per cent., and in maculo-anaesthetic and nervous ones 65·6 and 64·8 per cent. positive reactions. In suspicious cases 54·5 per cent., in carriers 31·8 per cent. and in contacts 42·6 per cent. were positive, but none of 5 controls. In 154 other cases, mostly skin troubles, 30 reacted including tuberculosis and acne patients. Activation by 2 grains of potassium iodide daily for a week increased the reactions in 88 doubtful cases by 31, but some positive cases became negative after KI. In many positive contacts acid-fast bacilli were found in glands by puncture. *L. R.*

i. SÉZARY (A.), LÉVY (Georges) & BOLGERT (M.). L'action thérapeutique du vaccin antilépreux de Vaudremer. [**Therapeutic Action of Vaudremer's Antileprotic Vaccine.**]—*Bull. et Mém. Soc. Méd. Hôpit de Paris.* 1934. Nov. 5. 50th Year. 3rd Ser. No. 27. pp. 1372–1381.

ii. SPITZER. Traitement de la lèpre par le vaccin de Vaudremer.—*Ibid.* Nov. 12. No. 28. pp. 1390–1391.

i. This vaccine is prepared by cultivating sterile fragments of leprosy nodules on an aspergillus medium, and what are considered to be evolutionary forms of the lepra bacillus are subcultivated on gelatine and sterilized by iodine to form the vaccine. Three cases treated by this vaccine are recorded with some improvement.

ii. This is a report on a single case treated with 26 injections of Vaudremer's vaccine in the course of six months with diminution of the discoloration and pigmentation of the skin and disappearance of infiltration of the leg. *L. R.*

i. SOUCHARD (L.). Dix-huit mois de fonctionnement d'un dispensaire antilépreux à l'Institut Pasteur de Saigon. Traitement par le savon total de krabao. Considérations sur la prophylaxie de la lèpre. [**Treatment by Krabao Soap in Indo-China.**]—*Arch. Insts. Pasteur d'Indochine.* 1933. Oct. No. 18. pp. 267–277. With 11 figs. on 4 plates.

ii. —— & RAMIJEAN. Contribution à l'étude du traitement de la lèpre par les savons de "krabao." Résultats constatés après un an de traitement chez des malades anciens internés à la Léproserie de Cu-Lao-Rong.—*Ibid.* pp. 187–265.

iii. GUILLERM (J.), BANOS (M.) & NGUYEN-VAN-LIEN. L'utilisation du "krabao" Indochinois pour le traitement de la lèpre.—*Ibid.* pp. 171–185.

iv. PERROT (Em.). Les espèces chaulmoogriques et, en particulier, le Krabao indochinois pour le traitement de la lèpre.—*Bull. Acad. Méd.* 1934. Nov. 20. 98th Year. 3rd Ser. Vol. 112. No. 37. pp. 602–605.

i. This paper records the well-known vegetable sources of chaulmoogra oils containing chaulmoogric and hydnocarpic acids, and the methods of preparing their therapeutic products used in French Eastern possessions. The necessity for obtaining fresh seeds of *Hydnocarpus wightiana* in India and *Hydnocarpus anthelmintica* in Indo-China is pointed out, from which the oil is obtained by pressure in the cold. In the preparation of the soaps or sodium salts the importance of neutralizing them with carbonate of soda is emphasized and also their preservation in a dry state protected from humidity.

ii. A trial of the soaps orally in 48 advanced voluntary cases of from ten to twenty years' duration in the Cu-Lao-Rong leper asylum, where no early cases were available, is recorded with full notes. The drug was given in 0·30 centigram pills, of which up to twenty were taken daily for five months, and the course repeated after two months interval, and a third course given after one and a half to two months further rest. A month after the commencement of the treatment most of the patients showed diminution of their nerve pains, but only after three months treatment was improvement in the cutaneous lesions observed in some only of the cases. Most patients only tolerated 14 to 16 pills daily

and minor digestive troubles were noted in many and considerable hepatic deficiency in some. Among 42 patients who persisted with the treatment decided amelioration was noted in 14, or 34 per cent., doubtful improvement in 6, or 15 per cent. no change in 11, or 26 per cent., and in 11 or 26 per cent. retrogression was noted. The chief difficulties in the treatment were due to the very advanced nature of the cases, with gastric intolerance in some, and associated syphilis, tuberculosis and other complicating diseases in others. The authors conclude that in about 30 per cent. of these 10 to 20 year old cases amelioration was obtained, but that by the treatment of such alone leprosy cannot be controlled; prophylactic measures are necessary, although very difficult to carry out in Indo-China.

iii. This paper deals with a trial of the soaps orally in an out-patient clinic which 67 patients attended, but only 47 at all regularly. The results were very similar to those above described in the asylum cases. Photos show great improvement in a few of the nodular cases. The method is considered to be of considerable importance.

iv. This brief note also enumerates the chaulmoogra oil bearing trees and mentions the composition of *H. anthelmintica* or Krabao oil.

L. R.

PAGET (H.), TREVAN (J. W.) & ATTWOOD (A. M. P.). **The Irritant Constituent of Anti-Leprotic Oils.**—*Internat. Jl. Leprosy.* Manila. 1934. Apr.–July. Vol. 2. No. 2. pp. 149–158. [10 refs.]

During an investigation for irritant properties of the still unidentified constituents of the total fatty acids of *H. wightiana* and sapucainha oils separated by the cold process, the authors found that the only such product was a 9 per cent. tarry fraction, which appeared to consist essentially of a lactonic acid. The ethyl esters of the crystalline acids were not rendered irritant by distillation at 350°/760 mm., but became so on long exposure in thin layers to light and air, possibly due to the production of lactonic acid. *L. R.*

EMERSON (George A.). **Mechanism of the Emetic Action of the Chaulmoograte.**—*Proc. Soc. Experim. Biol. & Med.* 1934. Oct. Vol. 32. No. 1. pp. 238–240.

As READ has cast doubt on his own evidence as to the central emetic action of chaulmoograte, the author has reinvestigated the matter by feeding dogs and cats, and he confirms the central action of the drug. He also found that cannabis, atropine and morphine all act in abolishing the emetic response in these animals. *L. R.*

NOLASCO (J. O.). **Histologic Studies on the Plancha or Infiltration Method of Leprosy Treatment.**—*Internat. Jl. Leprosy.* Manila. 1934. Apr.–July. Vol. 2. No. 2. pp. 159–174. With 2 plates. [22 refs.]

This is a useful summary of the author's histological investigations of the effects on the tissues of the injection of chaulmoogra oil preparations in the case of lepers, non-lepers, dogs and monkeys. The ethyl esters induce a mild inflammatory reaction with an accumulation of

the drug, especially as yellowish globules in large mononuclear cells, which may persist for nine months with beneficial results. Sodium hydnocarpate and alepol cause very similar reactions together with thrombosis of the larger local vessels which limit their use, and their irritant effects appear to reside in their fatty acid radicle. Tests on monkeys indicate absorption of the drugs through the lymphatics where they may reach the lepra bacilli. Injections of the whole oil cause less cellular reaction than iodized esters. The nerve trunks were found to be unaffected. The histological changes are illustrated by camera lucida drawings. *L. R.*

NOLASCO (J. O.). **Local Effects of Injection of Iodized Wightiana Ethyl Esters and Wightiana Oil around Nerve Trunks.**—*Jl. Philippine Islands Med. Assoc.* 1934. Nov. Vol. 14. No. 11. pp. 421–433. With 7 figs. [13 refs.]

This study was made on monkeys killed at various intervals after the injection of esters and wightiana oil around nerve trunks, which produced acute inflammatory reactions with fibro-cellular exudates and even pus formation. The material was phagocytosed by large mononuclears and carried up and down the limb in the loose perineural tissues, but was never found within the nerve capsule. Slight leucocytic infiltration within the sheaths indicated extension of the inflammatory process; to this the beneficial results in the relief of pains and anaesthesia after the injections is attributed. *L. R.*

LABERNADIE (V.). Essais de traitement de lépreux par des injections intraveineuses d'huile de chaulmoogra (résultats obtenus après 6 mois de traitement). [**Treatment by Intravenous Injections of Chaulmoogra Oil.**]—*Ann. de Méd. et de Pharm. Colon.* 1934. July–Aug.–Sept. Vol. 32. No. 3. pp. 328–337.

The results of six months treatment by intravenous injections of *H. wightiana* oil given very slowly through a fine needle are reported. One cc. doses twice a week were used, either exactly neutralized or with the slight acidity of 3 per 100 oleic acid. No coughing or other trouble occurred after the injections nor any febrile reactions or obliteration of the veins. Both preparations gave very similar results and isolated congested nodules benefited more rapidly, while pigmentation and anaesthesia recovered more slowly, but atrophic lesions did not improve. *L. R.*

MONTEL (L. R.). Traitement de la lèpre par le bleu de méthylène en injections intraveineuses. [**Treatment of Leprosy Intravenously by Methylene Blue.**]—*Bull. Acad. Méd.* 1934. Oct. 2. 98th Year. 3rd Ser. Vol. 112. No. 30. pp. 208–230. [13 refs.]

This important paper records the results of nine months trial in 172 lepers of the author's method of intravenous injections of 1 per cent. neutral methylene blue sterilized by heating on three consecutive days for one hour at 80°C., at which temperature febrile reactions are less frequent than at 120°C.

The first dose is 5 cc. increased at each injection to the limits of tolerance, which is usually between 25 and 35 cc., and given three times

a week up to 18 doses, and the courses repeated after intervals of 20 days. All the dermal leprous lesions retain the dye and stand out clearly so that the injection may be of diagnostic value as regards slight lesions. The first effect is the cessation of neuralgic pains with improved sleep and appetite and general condition. In a few cases fever results with congestion of the skin lesions followed by retrogression. Further extension of the lesions is arrested and thickening and oedema subside. The drug does not cause albuminuria. Recent lesions subside first with intense desquamation of the skin. Infiltrated patches may take four months for the thickening to disappear and the older lesions may take still longer, especially actual nodules, and after eight months treatment only partial subsidence of old fibrous nodules has been observed. On the other hand, ulcers, blisters, etc., heal rapidly and cease to discharge lepra bacilli and perforating ulcers are also very beneficially affected, nine out of ten such ulcers having healed in from two weeks to three months. Equally important is the frequent cessation of the discharge of lepra bacilli from the nose, greatly diminishing the infectivity of the patients. In nerve cases, in addition to the cessation of pain, the extension of the skin lesions is arrested and the thickened edges of tuberculoid lesions and thickening of superficial and trunk nerves subside. The treatment has thus been beneficial in all types of leprosy and it can be combined with chaulmoogra oil medication during the intervals in the methylene blue injections with advantage. *L. R.*

i. Freville (L. H. F.). Recherches expérimentales sur les réactions produites par les injections intraveineuses de bleu de méthylène dans la lèpre. [**Reactions produced by Intravenous Injections of Methylene Blue.**]—*Bull. Soc. Méd.-Chirurg. Indochine.* 1934. Aug.–Sept. Vol. 12. No. 7. pp. 615–621. With 1 chart.

ii. Montel (M. L. R.). Rectification de priorité à propos du bleu de méthylène dans le traitement de la lèpre. [**Priority in the Treatment.**]—*Ibid.* p. 622.

iii. ——. Traitement de la lèpre par le bleu de méthylène en injections intraveineuses.—*Ibid.* pp. 623–646. [13 refs.]

iv. Bigot (A.) & Le-van-Trien. Trois cas de lèpre traités par la méthode de Montel, au bleu de méthylène.—*Ibid.* pp. 734–739.

i. The paper of Freville deals with the reactions met with after intravenous injections of methylene blue by Montel's method in 150 cases. Cardiac syncopal attacks were occasionally met with and were not prevented by adding adrenalin, so the injections should be given with the patient recumbent. Febrile reactions were also met with after the use of solutions sterilized at 80°C., but solutions prepared without heat by the use of previously sterilized materials caused very little reaction of any kind. The drug can also be given in a 1 in 20 solution intramuscularly, which produces blue staining of dermal lesions more slowly than after their intravenous use.

ii. In this brief note Montel points out that G. A. Ryrie tried intravenous injections before him, but did not apparently note benefit from methylene blue as it is not one of the dyes he advises in leprosy treatment.

iii. The third of these papers is the same as that reviewed above.

iv. Brief notes of three cases treated with immediate benefit by Montel's method, which the authors propose to follow up. *L. R.*

i. MONTEL (M. R. L.). Les critères cliniques de l'action des traitements antilépreux. [**Methylene Blue Treatment.**]—*Bull. Soc. Méd.-Chirurg. Indochine.* 1934. June–July. Vol. 12. No. 6. pp. 559–565.

ii. —— & TRUONG-VAN-QUE. Un cas de lèpre généralisée à poussées aiguës traité par le bleu de méthylène. Observation et bilan après 153 jours de traitement.—*Ibid.* pp. 566–582. With 4 figs.

iii. DOROLLE (P.), NGO-QUANG-LY & TRAN-VAN-TAM. Premiers résultats dans le traitement de la lèpre par le bleu de méthylène (méthode de M. L. R. Montel).—*Ibid.* pp. 609–611.

i. This paper repeats points already dealt with above.

ii. This is a detailed account of the treatment of a girl of 17 with extensive mixed lesions, which greatly improved after 153 days of injections of methylene blue alternating with chaulmoogra preparation.

iii. Four cases of leprosy benefiting much from methylene blue treatment and confirming MONTEL's work. *L. R.*

AFANADOR (A.). Traitement de la lèpre par les injections intraveineuses de bleu de méthylène. [**Methylene Blue Treatment.**]—*Bull. Soc. Path. Exot.* 1934. Nov. 14. Vol. 27. No. 9. pp. 805–806.

This is a brief report on 20 cases of leprosy treated by Montel's method in a sanatorium at Valbonne by the injection intravenously of a total of 280 cc. of the 1 per cent. solution in 30 days giving 3 injections weekly. The immediate effects were similar to those described by MONTEL, but the time under observation was too short to allow of any modification of the zones of anaesthesia and atrophy. *L. R.*

LEGGATE (James). **Bonney's Blue Solution in the Treatment of Leprosy.**—*Leprosy Review.* 1934. Oct. Vol. 5. No. 4. pp. 161–162.

The author has used this treatment with success in ten advanced cutaneous cases of leprosy. Brilliant green and crystal violet, of each 0·5 gm. in absolute alcohol 25 cc. and distilled water 2,500 cc., sprayed on leprous ulcers, and lint soaked with it applied at night, removed suppuration and induced healing. Intradermal injections of the solution were less rapidly beneficial in hard nodules and more quickly so in soft ones, followed by its intravenous use, alternating with iodized esters. In nerve cases the results were not so striking, but laryngeal, throat and nose conditions have responded well. *L. R.*

RYRIE (Gordon A.). **On the Use of Fluorescein and Phthallic Acid in Leprosy.**—*Internat. Jl. Leprosy.* Manila. 1934. Apr.–July. Vol. 2. No. 2. pp. 139–147.

The author records that the most stable results obtained from the intravenous injection of various aniline dyes followed the use of fluorescein in sixty-four cases treated for four months. In about 60 per cent. clinical and microscopical examinations of the lesions showed a greater or less specific response, but the improvement rate dropped to 43 per cent. on continuing the treatment for four months. In order to ascertain which constituent of the dye was effective nine cases were treated with one of its constituents, resorcin blue, with negative results, but in 16 patients treated for two months with intravenous injections of another constituent, phthallic acid, greater or less response was observed in 62 per cent., so this is considered to be the active part of

the dye. It is noted that hydnocarpus esters appear to be better tolerated after a course of fluorescein, but that very little evidence was obtained of the beneficial effect of the dye in advanced cases of leprosy.

L. R.

EMERSON (George A.) & ANDERSON (Hamilton H.). **Toxicity of Certain Proposed Antileprosy Dyes : Fluorescein, Eosin, Erythrosin, and Others.**—*Internat. Jl. Leprosy.* Manila. 1934. Aug.–Oct. Vol. 2. No. 3. pp. 257–263. [16 refs.]

The authors state that possibly dangerous doses of certain dyes have been used by RYRIE intravenously in leprosy, so their toxicity has been investigated with the following results.

" Fluorescein, eosin, erythrosin and methylene green were found to be lethal at 300, 350, 200 and 150 mgm. per kilogram, respectively, when administered intravenously to rabbits, and at 600, 500, 300 and 125 mgm. per kilogram intraperitoneally in rats. Methylene green is lethal for anesthetized cats in doses of 50 to 75 mgm. per kilogram. Orally in rats these dyes are tolerated in doses of 1·0 gm. per kilogram with the exception of methylene green, which killed 2 of 5 rats at 500 mgm. per kilogram. Data are presented on the chronic toxicity of trypan blue, gentian violet, brilliant green and mercurochrome. Three of 6 rabbits dying under repeated intravenous administrations of trypan blue had received a total cumulative dose, approximately equivalent to but one acute lethal dose, *i.e.*, 120 to 150 mgm. per kilogram. The dangers of repeatedly using high doses in human lepers, the superiority of oral administration over intravenous, and the danger of certain synergizing agents, including photodynamic effects are discussed." *L. R.*

PEREIRA (O. Loyola). **On the Effects of Anti-Variolic Vaccination in Lepers.**—Reprinted from *Antiseptic.* 1934. July. 4 pp. With 1 plate.

This is a brief description of the well-known severe febrile and local reactions often following vaccination of lepers against smallpox ; 15 of 24 vaccinated lepers showed such reactions with great debility. *L. R.*

PALDROCK (A.). Durch spezifische Behandlung von Lepra geheilt. [**Leprosy cured by Specific Treatment.**]—*Arch. f. Schiffs- u. Trop.-Hyg.* 1935. Jan. Vol. 39. No. 1. pp. 23–25. With 1 fig.

This is a short account of a further case of leprosy treated successfully by the author's method of local applications to the skin lesions of carbonic acid snow and injections of the gold preparations solganal and lopion.

L. R.

WATANABE (Y.). **Experimental Studies on the Lepra Bacillus. (Part I.) Inoculation Test with the Bacillus of Rat Leprosy. (Part I.)**—*Kitasato Arch. Experim. Med.* 1934. Oct. Vol. 11. No. 4. pp. 259–276.

After references to earlier Japanese work the author records his own experiments on rats and white mice by inoculation and ingestion of the rat leprosy bacillus. The results in rats were very similar to those of previous workers, local lesions developing at the site of subcutaneous injection in two weeks to three months and inconstant extension to internal organs. Orally infection was slight without involvement of the mesenteric glands. In the mice subcutaneous inoculation

only led to enlarged axillary glands containing acid-fast bacilli. Intravenous injection also showed slighter changes in the internal organs than in rats, but intraperitoneal injection involved the mesenteric glands, liver and spleen, and subinoculation caused similar changes.

L. R.

BERNY (P.). Echec de la transmission aux lapins et aux cobayes des bacilles de la lèpre des rats. [**Bacilli of Rat Leprosy not Transmissible to Rabbits and Guineapigs.**]—*Bull. Soc. Path. Exot.* 1934. Oct. 10. Vol. 27. No. 8. pp. 717–719.

The author reports the production in rabbits and guineapigs injected with rat leprosy bacilli of thickening, sometimes going on to abscess formation before resolving. Acetone extracts may also produce abscesses at the site of injection, in some of which tubercle bacilli were found. Phagocytes containing acid-fast bacilli were also met with, but bacilli taken from animals on the 74th day and injected into rats disappeared without producing infection. *L. R.*

ATKEY (O. F. H.). The Distribution of Leprosy in the Sudan with Reference to Climate and Diet.—*Internat. Jl. Leprosy.* Manila. 1934. Apr.–July. Vol. 2. No. 2. pp. 193–200. With 1 map in text. [See this *Bulletin*, Vol. 31, p. 542.]

GUERRIERI (Tito). Contributo allo studio sulla lebbra dei ratti.—*Arch. Ital. Sci. Med. Colon.* 1934. Nov. 1. Vol. 15. No. 11. pp. 801–827. With 4 figs. [22 refs.] English summary (6 lines).

MENDIOROZ (Julio). Estado de la lepra en Salta en 1933.—*Prensa Méd. Argentina.* 1934. Dec. 19. Vol. 21. No. 51. pp. 2415–2423. With 3 figs.

SLEEPING SICKNESS.

DUREN (A.) & VAN DEN BRANDEN (F.). Sur un cas de trypanosomiase humaine à évolution latente. [**Sleeping Sickness of Slow Evolution.**]—*Ann. Soc. Belge de Méd. Trop.* 1934. Dec. 31. Vol. 14. No. 4. pp. 437–438.

Details of two cases are given. A European who had left the Congo 7 months previously and had not complained of any symptoms was found by chance to have an erythematous eruption, enlarged glands, with trypanosomes in the blood and gland juice. The cerebrospinal fluid showed considerable changes. This was a case which was latent from the point of view of subjective symptoms, but of normal evolution.

The second case was, however, more characteristic. The authors were consulted in May, 1934, by a man who had returned to Belgium from the Congo in November, 1932. During the first few months after his return from the tropics he suffered from slight fatigue, but improved with Fowler's solution. In November, 1933, he again felt tired, but did not consult a doctor. When he was seen by the authors in May, 1934, he was more definitely fatigued and had tachycardia. No other clinical sign or symptom was discovered, but trypanosomes were found in the blood. Careful interrogation indicated that the patient was probably infected in April 1932, that is 25 months before a diagnosis was made, and 20 months before there were any disquieting symptoms. A course of 3·5 gm. of Bayer and 21 gm. of tryparsamide was given At the end of July, 15 days after cessation of treatment, spinal puncture revealed a normal cerebrospinal fluid. *W. Yorke.*

SICÉ (A.) & MERCIER (H.). Trypanosomiase nerveuse et tuberculose. A propos d'un double échec de la cure tryparsamique. [**Nervous Trypanosomiasis and Tuberculosis.**]—*Bull. Soc. Path. Exot.* 1934. Dec. 12. Vol. 27. No. 10. pp. 924–929.

Details are given of two advanced cases of sleeping sickness in which treatment by tryparsamide caused latent tubercular lesions to light up with the production of severe general symptoms. *W. Y.*

BERTRAND (Ivan), BABLET (J.) & SICÉ (A.). Lésions histologiques des centres nerveux dans la trypanosomiase humaine (à propos de deux cas mortels non traités). [**Histological Lesions of the Central Nervous System in Human Trypanosomiasis.**]—*Ann. Inst. Pasteur.* 1935. Jan. Vol. 54. No. 1. pp. 91–144. With 13 figs. & 3 plates. [52 refs.]

A detailed account is given of the histological changes found in the central nervous system of two untreated cases of sleeping sickness.

The paper opens with a lengthy résumé of the work of previous investigators, and this is followed by a detailed history of the two cases examined. The history and the macroscopic findings at the post-mortems showed that the two cases differed considerably. The pathological changes in the first case were much more pronounced, and there was unquestionable evidence of a diffuse leptomeningitis, whilst in the second case the leptomeningitis was scarcely noticeable.

It is almost impossible to give an adequate summary of the lengthy description of the histological findings, and the paper must be consulted in the original by those interested.

The following are the authors' conclusions :—

From the very first sleeping sickness is a diffuse meningo-encephalitis, the infiltrative character of which is very marked. The perivascular lesions exhibit a predominance of plasma cells not encountered in any other infection. The white matter is particularly rich in vascular lesions. There is no question, however, of a leuco-meningitis analogous to that seen in Schilder's disease.

The cellular elements of the infiltrations have a complex origin. In addition to strictly nervous and microglial elements there are a large number of plasmocytes and of histiocytes of adventitious or meningeal origin. The infiltrations are then partly glial and partly mesenchymatous.

The morula cells of Mott are strictly identical with the fuchsinophil cells of Russell. Without being able to state the exact chemical constitution of their inclusions, there is no doubt that they are derived exclusively from the plasmocytes. The neuroglia cells never exhibit inclusions of this kind. The histiocytes and the microgliocytes are able to engulf the bodies of morula cells when they have reached a sufficiently advanced degree of disintegration.

The neuroglial formula of trypanosomiasis depends essentially on the length of its evolution. The presence of numerous cells *en bâtonnet* of Nissl in the substance of the grey cortex and even in the white matter indicated a chronic process, but is in no way pathognomonic.

The numerous amoeboid cells of the neuroglia, which are seen in the white matter, sometimes in great number, in preparations impregnated with gold sublimate (Ramon Cajal) show prolongations, hypertrophied but free, contrary to the classical opinion.

Clasmatodendrosis is rare and generally limited to the suckers of the fibrous neuroglia.

The cortical glia is much less attacked than in general paralysis.

The neuroganglion lesions of the cerebral cortex consist chiefly of an acute tumefaction. The appearances of liquefaction indicative of a grave and irreversible degeneration are absent, in contrast to what is seen in paralytic dementia. *W. Y.*

MACKIE (F. P.). **The Jarisch-Herxheimer Reaction in Trypanosomiasis. With a Note on the Morular Cells of Mott.**—*Trans. Roy Soc. Trop. Med. & Hyg.* 1935. Jan. 25. Vol. 28. No. 4. pp. 377–384. With 2 plates. [25 refs.]

An account is given of the post-mortem changes found in the brains of two men who died of *rhodesiense* sleeping sickness. Each exhibited features which justify a special note, in the first regarding the Jarisch-Herxheimer reaction, and in the second the " morular bodies of Mott."

Case I.—Contracted sleeping sickness in Rhodesia about a year previously, but, as the nature of the illness had not been suspected, he had been given no specific treatment until a few weeks before death, when he received 3 doses (total 1 gm.) of N.A.B. on another assumption. On admission to hospital *T. rhodesiense* was scanty in the blood and cerebrospinal fluid. The day following admission the patient was given an intravenous injection of tryparsamide, grams 2. The temperature, which was high at the time, continued to rise and reached 107·2°F. an hour before death. The patient became comatose, with marked tremor of the right leg and arm ; nuchal rigidity and Kernig's sign were present ; and the pupils were dilated and fixed. Lumbar puncture was performed. The cerebrospinal fluid was clear, but the pressure was increased, as were also the globulin and lymphocyte contents.

Post-mortem.—A detailed account is given of the findings at the autopsy. On opening the skull there was a gush of blood-stained cerebrospinal fluid. The membranes were congested; there was a considerable amount of blood-stained oedema over the Rolandic areas on the left side, and there was gelatinous oedema under the pia over the vertex. On cutting through the base of the ganglia there was seen to be a wide area of deep red haemorrhagic softening occupying the anterior half of the caudate nucleus and the anterior and lateral aspects of the lenticular nucleus. These appearances were due to the confluence of punctate haemorrhages producing a massive effect.

Case 2.—This was a chronic case infected in Northern Rhodesia in 1928. The patient had suffered from five relapses and had received, during the course of his illness, 36 grams of Bayer 205 and 33 grams of tryparsamide.* The patient failed to respond to treatment and death occurred without any signs of a cerebral catastrophe, such as occurred in the former case.

A detailed account of the post-mortem findings is given.

In discussing the Jarisch-Herxheimer reaction, the author points out that this phenomenon was first described by JARISCH (1895) as an exacerbation of cutaneous syphilides resulting from mercurial treatment. HERXHEIMER and KRAUSE (1902) showed that the reaction was not confined to cutaneous manifestation, but was a systemic reaction, which caused a general "flare-up" of syphilitic activity, wherever such lesions were present, and that it was much more common and severe as the result of treatment with organic preparations of arsenic. Clinically, the reaction may be nothing more than a slight rise of temperature with an exacerbation of local signs, or there may be alarming symptoms, such as excruciating headache, vomiting, tremors, epileptic convulsions, coma, and death.

Autopsy in such cases may reveal nothing more than vaso-dilatation of the cerebral meninges with irregularly distributed areas of cerebral softening. In some cases these were associated with punctate haemorrhages or small focal haemorrhages in various parts of the cerebrum. The various hypotheses which have been advanced to explain the Jarisch-Herxheimer reaction are briefly discussed. There is no doubt that a genuine Jarisch-Herxheimer reaction occurs in human sleeping sickness, but this reaction is confused or over-shadowed by the more frequent therapeutic catastrophes which are almost certainly due to toxic alterations in the drugs used.

Morula cells.—These were first described by MOTT (1906) as follows:—"... large, round or oval cells with the nucleus staining deep blue and pushed up to one end or pole; the cytoplasm consisting of a number of clear spherules, staining by eosin giving the cell a mulberry appearance: hence I have called these cells 'morular cells'. They correspond to the Körnchenzellen of Alzheimer. The appearance of these cells suggests degenerated plasma cells. Similar cells are seen in the degenerated structures of infected lymphatic glands."

PERUZZI has given a full account of their distribution, structure and probable origin. He describes them as cells with fuchsinophile hyalin globules, which after destruction of the protoplasm and rupture of the cell present all the characters of Russell's bodies. He thinks that though they may have a multiple origin in trypanosomiasis, they are generally derived from the neuroglia, and he believes that they are indicative of the presence of a severe virus causing nerve cell destruction.

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* The original has "grains" in each instance; a correction is published in the succeeding number of the Transactions.

In Mackie's case morula cells were most numerous in the cellular exudate of the pia and in the perivascular cuffs, but they were also seen in the brain substance, not obviously in connexion with blood channels. They are also found occasionally in other organs, and similar bodies have been seen by Mackie in normal intestinal mucosa. This fact excludes the assumption that their origin is invariably from neuroglia cells. *W. Y.*

CORSON (J. F.). **Experimental Transmission of** *Trypanosoma rhodesiense* **through Antelopes and** *Glossina morsitans* **to Man.**—*Jl. Trop. Med. & Hyg.* 1935. Jan. 1. Vol. 38. No. 1. pp. 9–11.

A strain of *T. rhodesiense* taken from man a year previously was found to be still infective for man after a number of cyclical passages through *G. morsitans* and dik-diks. It has been previously shown by Corson that *T. rhodesiense* could be maintained in sheep and goats for nearly two years without losing its transmissibility by *G. morsitans* or its infectivity for man. In the present experiment, *T. rhodesiense* was transmitted by single isolated infective *G. morsitans* from antelope to antelope, and finally to man.

The experiments commenced on July 24, 1933, when the blood of a Rhodesian sleeping sickness patient was inoculated into a number of guineapigs. From one of these guineapigs a fly was infected, and this in due course infected a dik-dik. The strain was then passed through fly—dik-dik—fly—dik-dik—fly. During the year, therefore, the strain had passed through a guineapig, 4 tsetse flies, and 3 dik-diks. The last fly was fed on a volunteer on the 27th July, 1934. A ring was drawn round the site of the bite with silver nitrate. On the 5th day, a slightly red spot was noticed within the ring. On the 6th day the spot was about a $\frac{1}{4}$ inch in diameter and somewhat raised and congested, and the skin felt thickened. A stained thick blood film showed no trypanosomes; the patient felt well. On the 7th day the spot was $\frac{3}{4}$ inch in diameter and slightly painful and tender, and the blood was found to contain trypanosomes. *W. Y.*

CORSON (J. F.). **Resistance of White Rats to Infection with** *Trypanosoma rhodesiense* **through eating Infected Tissues of Rats.**—*Ann. Trop. Med. & Parasit.* 1934. Dec. 20. Vol. 28. No. 4. p. 589.

After referring to the work of DUKE, METTAM, and WALLACE [*ante*, p. 33] on the infection through the mucous membrane of the mouth by feeding kittens on the carcases of rats infected with *T. brucei*, Corson states it is important in experimental work to know whether such accidental infections may occur. He states that in his experiments during the last 8 years over 1,000 white rats have been infected with *T. rhodesiense* or *T. brucei*, but although a stock of about 1,000 rats is usually kept on the premises, no case of accidental infection has occurred.

An experiment is described in which various parts of the body, viz., liver, spleen, kidneys, heart, lungs, and hind-legs, of rats heavily infected with *T. rhodesiense* were given to healthy rats to eat. The work was done under careful supervision, each rat being able to eat its portion undisturbed. Of the 52 rats so fed, none became infected.

From this Corson concludes that it is unlikely that experiments with *T. rhodesiense* and white rats will be affected by accidental infection of the rats through eating the carcases of other rats. *W. Y.*

CORSON (J. F.). **The Influence of the Dose of Trypanosomes and of the Body Weight in Experimental Infections of White Rats with** *Trypanosoma rhodesiense.—Ann. Trop. Med. & Parasit.* 1934. Dec. 20. Vol. 28. No. 4. pp. 525–534. [13 refs.]

Results are recorded in this paper of a considerable number of observations designed with the object of ascertaining whether the number of trypanosomes injected, and the body weight of the animal, had any effect on the resulting infections of white rats with *T. rhodesiense.*

In the first experiments 10 rats, each weighing approximately 100 gm., and 10 weighing about 50 gm. each, were inoculated subcutaneously with 100,000 trypanosomes. The results, which are set forth in a table, show that the incubation period and duration of life are about the same in both series. A series of experiments were then performed with the object of ascertaining the influence of the number of trypanosomes injected. These vary from 100,000 to 100. The experiments show that within these limits the number of trypanosomes had little, if any, influence on the incubation period and the duration of the infection. The experiments show, however, that it is important to introduce a certain amount of serum into the diluting Ringer-glucose solution, as this solution, in the absence of protein, will not support trypanosomes for any length of time. [Attention has already been drawn to this fact by the reviewer and his colleagues.]

Corson points out that in his experiments with *T. rhodesiense* the bite of an infected *G. morsitans* has never failed to infect normal white rats, and the incubation period and duration of the disease have been very similar to those observed after infection with a syringe. A long series of observations on this point is summarized in a table. In 80 per cent. of the rats mentioned in this table the incubation period was either 4 or 5 days, and the duration of life was from 20 to 30 days. The variations in the duration of life can be explained by differences in the resistance of individual rats. *W. Y.*

LAUNOY (L.). Incubation clinique et pouvoir infectant du sang, dans l'infection expérimentale à *Trypanosoma congolense* du cobaye. [**Clinical Incubation and Infecting Power of the Blood in Experimental Infections of Guineapigs with** *T. congolense.*]—*C. R. Soc. Biol.* 1934. Vol. 117. No. 37. pp. 1047–1049.

In a previous communication the author has shown that the blood of guineapigs, infected by intraperitoneal inoculation of 3 or 4 million trypanosomes, was infective long before parasites could be discovered microscopically. He calls the period between inoculation and infectivity of the blood "bacteriological incubation," and that between inoculation and microscopical discovery of the parasites in the blood "clinical incubation." In certain cases, the former period was only 8 hours, whereas the latter was between 6 and 9 days.

In the present work the author has inquired whether the "bacteriological incubation" was modified by the number of trypanosomes introduced into the peritoneal cavity. The results of his experiments are summarized in tables. With two exceptions the blood of

every animal which had received 70,000 or more trypanosomes was infective within 24 hours. Of the seven guineapigs which received 11,750 trypanosomes, the blood of only one was infective under 30 hours, whilst in none of those which received only 1,175 trypanosomes was the blood infective within 24 hours. The author points out, however, that one must not conclude from this that the blood of guineapigs which have been given only 1,175 trypanosomes is really non-virulent after 24 hours. It takes about 2 hours to count the parasites and make the various dilutions, and during this period many of the trypanosomes are damaged by the physiological saline used for diluting. Nevertheless, this relatively small number of trypanosomes suffices to infect, and the " clinical incubation " is not very different from that observed after massive injections. *W. Y.*

LAUNOY (L.) & ANCELOT (A.). Sur le pouvoir infectant, après différents traitements, du sang de souris infectées par *Trypanosoma congolense.* [**The Infectivity after Different Treatments of the Blood of Mice infected with** *T. congolense.*]—*C. R. Soc. Biol.* 1935. Vol. 118. No. 4. pp. 328–330.

Groups of mice heavily infected with *T. congolense* were treated with Bayer 205, tryparsamide and the sodium salt of antimony thiomalate. At various periods, 2 to 25 hours, afterwards the blood was injected into healthy mice by one of the following three routes : intravenous, intraperitoneal, or subcutaneous. Of the mice injected intravenously all except one became infected, and the same applied in the case of those injected intraperitoneally ; but of the mice injected subcutaneously only 8 of 44 became infected. Very similar results were obtained from mice which received synergic treatment, *i.e.*, tryparsamide 0·06 gm. + Bayer 0·005 gm. or the antimony compound 200 γ + Bayer 0·005 gm.

The authors believe that this curious phenomenon is due to a particularly energetic defensive reaction, which is brought into operation on subcutaneous injection. *W. Y.*

VAN DEN BRANDEN (F.). Essais comparatifs du traitement des rats blancs infectés de *Trypanosoma congolense*, par l'orsanine sodique (270 Fourneau) et par le tryponarsyl. [**The Treatment of Rats infected with** *T. congolense* **by Orsanine and Tryponarsyl respectively.**]—*Ann. Soc. Belge de Méd. Trop.* 1934. Sept. 30. Vol. 14. No. 3. pp. 375–378.

It is well known that *T. congolense* infections are very resistant to arsenicals, and, therefore, in order to ascertain whether orsanine possesses a greater trypanocidal activity than tryponarsyl, the author has examined the effect of each on white rats infected with this parasite.

In the first experiment 6 infected rats weighing between 100 and 120 gm. were given 0·1 gm. of orsanine and 6 similar animals 0·2 gm. of tryponarsyl ; the rats given tryponarsyl were not sterilized, whilst those given orsanine were sterilized, but were poisoned by the drug and died within 36 hours. In a second series of observations the dose of orsanine was reduced to 0·05 gm. ; this dose was tolerated and caused the disappearance of trypanosomes from the blood for a period of 10 days. It follows that for white rats infected with *T. congolense* orsanine is more active than tryponarsyl. *W. Y.*

VAN DEN BRANDEN (F.) & POTTIER (R.). L'hexaméthylène tétramine associée à la tryparsamine dans le traitement de la trypanosomiase.—Contrôle biologique du tryponurile. [**Hexamethylene Tetramine (Urotropine) in Association with Tryparsamide in the Treatment of Trypanosomiasis.**]—*Ann. Soc. Belge de Méd. Trop.* 1934. Dec. 31. Vol. 14. No. 4. pp. 499–502.

In view of the statement of LIEURADE that the association of urotropine with tryparsamide increases the action of the latter in sleeping sickness [*ante*, p. 19], the authors have examined the point in rats infected with *T. congolense.*

Ten rats were given tryponarsyl alone, 0·25 gm. per 100 gm. of body weight; 10 other rats were given 0·25 gm. of urotropine followed 3 hours later by 0·25 gm. of tryponarsyl. No difference was noticed between the results given by the two forms of treatment. In a second group of experiments the procedure was similar, except that 0·5 gm. of tryponurile was given instead of the 0·25 gm. of urotropine. Tryponurile is a product of l'Union Chimique Belge, containing equal parts of tryponarsyl and hexamethylene tetramine. Here again no advantage occurred from the addition of urotropine. The authors accordingly conclude that hexamethylene tetramine does not re-inforce the action of tryponarsyl in *T. congolense* infections of rats. *W. Y.*

GOLDIE (H.). Effet du plasma, traité par le moranyl, sur la coagulation du sang. [**The Effect of Plasma treated with Moranyl in the Coagulation of the Blood.**]—*C. R. Soc. Biol.* 1934. Vol. 117. No. 33. pp. 677–681.

If 2 cc. of 0·9 per cent. solution of moranyl is added to 8 cc. of blood just removed from the vein of a horse, coagulation is certainly prevented, but 1 cc. to 1·5 cc. only prevents coagulation sometimes. When 2 cc. ("dose certaine") is used, the plasma remains fluid, and after 24 hours in the ice-chest can be removed from the red cells. Liquid plasma containing the smaller amount of moranyl, viz., 1 cc. to 1·5 cc. ("dose limite"), coagulates after the addition of thrombine, but the other factors concerned in coagulation, viz., fibrinogen, calcium and cytozyme, exert no influence on the plasma. The author then goes on to compare oxalated plasma with moranylized plasma; the paper is of a rather technical nature and should be consulted in the original by those interested. *W. Y.*

i. FISCHL (Viktor) & SINGER (Ernst). Die Wirkungsweise chemotherapeutisch verwendeter Farbstoffe. [**The Mechanism of Action of Chemotherapeutic Dyes.**]—*Ztschr. f. Hyg. u. Infektionskr.* 1934. Sept. 22. Vol. 116. No. 4. pp. 348–355. With 2 figs. [18 refs.]

ii. SINGER (Ernst) & FISCHL (Viktor). Weitere Versuche ueber die Wirkung von Arzneimitteln in vitro. [**Further Experiments on the Action of Drugs** *in vitro*.]—*Ibid.* pp. 356–360. [15 refs.]

i. In this paper the authors consider the mechanism of the action of certain dyes which are known to exert a trypanocidal activity. v. JANCSÓ has laid down that the trypanocidal activity of dyes is parallel with their absorption by the parasites and with their

photodynamic and *blepharocidal action [see this *Bulletin*, Vol. 29, p. 646]; in the case of resistant trypanosomes all four factors, cure, absorption, photodynamic action, and blepharocidal action, are inhibited.

After summarizing various observations which seem to throw doubt on the validity of v. Jancsó's hypothesis, the authors state that the necessity of re-investigating the mechanism of action of dyes is shown by the fact that they themselves have proved that in the case of metallic compounds the curative mechanism is a complex process consisting of 3 phases—(1) A physico-chemical adsorption of the substance by the cell of the pathogenic organism, (2) a change in this adsorbed substance as a result of the vital activities of the cell, resulting in the formation of an actual poison; and (3) finally the completion of cure by the immune substances of the organism of the host [this *Bulletin*, Vol. 31, p. 851]. It is a matter of importance to discover whether in the metal-free chemotherapeutic substances—of which the most characteristic are the dyes—the 1st and 2nd phases fall together or whether they are separate.

The most obvious criticism of the investigations of v. Jancsó and of those who followed him is that they failed to use suitable controls, *i.e.*, substances which not only exhibit the necessary fluorescence, but which, although chemically closely related to trypaflavin, have no therapeutic action. Atebrin fulfilled these conditions and was selected as the most suitable control substance. In a table the chemical constitution and the various physical properties (especially as regards fluorescence) of trypaflavin and atebrin are compared, and data are given regarding the doses—toxic, tolerated, curative, effective, etc.—of the two compounds for mice infected with nagana and birds infected with malaria.

Comparable investigations with the two dyes gave the following results:—

(*a*) Some minutes after treatment of a nagana mouse with the tolerated dose of trypaflavin the trypanosomes when examined by the usual dark-field method exhibited, as stated by v. Jancsó, strongly illuminated blepharoplasts; under "interference" conditions or with fast strains the phenomenon was not observed. After injection of the tolerated dose ($\frac{1}{200}$ gm.) of atebrin the trypanosomes were seen to be filled with numerous illuminated particles, but after injection of $\frac{1}{2000}$ gm. (*i.e.*, the tolerated dose of trypaflavin) nothing unusual was seen. This observation shows the specific affinity of trypaflavin for the blepharoplast and the storage of the inactive atebrin in the protoplasm of the parasites. But it cannot be decided from this whether the illumination of the blepharoplast is due to the elective storing of the drug in this structure or whether it is due to irritation. The authors do not believe that the blepharocidal action can be identical with the curative action.

(*b*) Observation of coverslip preparations, or dry smears of the blood, with the fluorescence microscope shows that both the active trypaflavin and the inactive atebrin are stored in the whole trypanosome body in exactly the same way.

(*c*) If the trypanosomes from the total blood of a mouse were collected 20 mins. after a dose of trypaflavin they were found to be coloured deep yellow, whilst after atebrin they were just as white as from an untreated animal. The fluorescence microscope, however, shows that this is only due to the weaker intensity of the atebrin stain.

These observations show that with trypaflavin, and the metal-free chemotherapeutic substances, the binding of the drug on the parasite

* A convenient abridgment of Blepharoplasticidal.

is indeed an indispensable procedure for specific action, but that it is not identical with it. This agrees with what the authors have previously found with the metallic compounds.

The authors object to the expression "lethale Lichtzahl" used by VON JANCSÓ in his photodynamic studies. They state that there is no relationship between the motility and infectivity of a trypanosome; and consequently it would be more correct to speak of "immobilisierende Lichtzahl." An experiment was conducted to ascertain whether the trypanocidal action *in vitro* of trypaflavin was only due to a photodynamic cause. The whole experiment was conducted in the dark. Trypanosomes from mice infected respectively with the normal and trypaflavin-resistant strains were kept *in vitro* in $\frac{1}{20000}$ solution of trypaflavin. After 15 minutes the normal trypanosomes were completely immobilized and granular, whilst the resistant parasites were unchanged and feebly motile; 0·2 cc. of each suspension was then injected into healthy mice. Under these conditions, in which light was almost completely excluded, neither the normal nor the resistant strain produced infection. In a similar experiment with atebrin both normal and fast strains exhibited normal appearances and motility after 15 minutes, but they displayed a markedly decreased infectivity.

The authors believe that from the experiments they have succeeded in demonstrating that curative action, trypanocidal action *in vitro*, and photodynamic action are completely independent of one another; the sole common factor in these phenomena and in blepharocidal action is the previous incorporation of the substance in the body of the parasite.

Analogous experiments were performed with atebrin and trypaflavin in bird malaria, and also with atebrin in human malaria.

ii. In this paper the authors have attempted to go further into the question whether any relationship exists between absorption of drugs by parasites and their destruction. In a table it is shown that if equal quantities of *Spirochaeta recurrentis*, *Proteus* sp., erythrocytes, collodion and animal charcoal are placed in a solution of 0·1 per cent. atoxyl for 1 hour they absorb considerably less arsenic than if they were placed in a 0·1 per cent. solution of atoxyl which had previously been digested with liver.

The next question which was investigated was the constituent of liver, which is responsible for this activating action on atoxyl and similar phenyl arsinic acids. In addition to cystin (not cystein) and those substances, viz., glutathion and glycogen in which liver is rich, the authors investigated thioglycollic acid and sodium thioglucose, and also mouse red cells and, on account of its high glutathion content, an emulsion of the calf's eye lens. Of all these substances it was found that glutathion alone exerted the activating influence on atoxyl for trypanosomes and spirochaetes. Other reducing substances known to be in the animal body are ergothionin, Vitamin B_2 and ascorbic acid (Vitamin C).

Experiment showed that ascorbic acid (1 : 800) killed trypanosomes *in vitro*, but had no action on spirochaetes. A mixture of atoxyl and ascorbic acid damaged the trypanosomes, but did not completely destroy them, whereas a mixture of sulfoharnstoff and ascorbic acid destroyed both trypanosomes and spirochaetes. In the authors' opinion these observations furnish additional evidence that the activity or inactivity of a drug *in vitro* has nothing to do with its activity or inactivity *in vivo*. *W. Y.*

FISCHL (Viktor) & FISCHL (Lili). Arzneifestigkeit, Avidität, Interferenz. [**Drug-Resistance, Avidity and Interference.**]—*Ztschr. f. Immunitätsf. u. Experim. Therap.* 1934. Sept. 18. Vol. 83. No. 3/4. pp. 324–335. [21 refs.]

This paper, which is of a rather technical nature, describes experiments dealing with the subject of drug-resistance.

It is recalled that SCHLOSSBERGER and MENK had recorded that a strain of nagana-Prowazek, which had been made resistant in mice to a trypanocidal gold derivative called "Sulfoharnstoff" proved to be sensitive to tartar emetic and trypaflavine, but definitely resistant to neosalvarsan and germanin; on the contrary, the same parent strain made resistant to neosalvarsan was found to be completely resistant to the arsenicals, somewhat resistant to tartar emetic, but normally sensitive to germanin and "Sulfoharnstoff."

The authors repeated this work with the same parent strain, but contrary to SCHLOSSBERGER and MENK found that the "Sulfoharnstoff" resistant strain was sensitive to neosalvarsan, trypaflavine and germanin. This "Sulfoharnstoff-fast" strain was then made resistant to trypaflavine. The sensitiveness of the strain to a large number of substances was then tested and the results given in a table. There follows a discussion of the significance of these results. It is especially difficult in drug-resistance experiments to understand why certain substances are almost always active (avid substances of EHRLICH), whilst other closely related derivatives exert no influence on the trypanosomes. Examples of such avid compounds are arsenophenylglycine, arsenophenoxy acetic acid and arsenophenylthioglycolic acid (EHRLICH) and the arsinic acid corresponding to arsenophenylglycine (YORKE and his collaborators). SCHNITZER recently has stated that myosalvarsan and solusalvarsan are avid compounds, but so far as myosalvarsan is concerned, this is not confirmed by the authors' experiments. As regards its avidity-index (SCHNITZER) or its resistant-factor (Fischl and SINGER) solusalvarsan stands midway between salvarsan and arsenophenylglycine.

The last portion of the paper is concerned with the chemotherapeutic interference phenomenon. It was found that ascorbic acid (Vitamin C) exercised a definite "interference" action against tartar emetic, arsenophenylglycine, m.amino-p-oxyphenyl-arsenoxide and trypaflavine. Details of the experiments are given in a table. *W. Y.*

VON JANCSÓ (Nikolaus) & VON JANCSÓ (Hertha). **The Rôle of the Natural Defence Forces in the Evolution of the Drug-Resistance of Trypanosomes. I.—A Method for the Exclusion of the Natural Defence Mechanisms from Chemotherapeutic Processes.**—*Ann. Trop. Med. & Parasit.* 1934. Oct. 19. Vol. 28. No. 3. pp. 419–438. [29 refs.]

The authors have devised a method which they claim enables them to exclude almost entirely the natural protective mechanism in rats and mice in trypanosome infections. The method consists essentially in splenectomy and in the intravenous injection of an electrically prepared colloidal copper solution.

This preparation was obtained from von Heyden and has 0·06 per cent. of copper content. Apparently the amount of copper, however, varies somewhat in different samples. With the latest solution supplied it was possible to produce a typical blockade by injecting 0·02 cc.

of the solution diluted with 4 times its volume of water, whereas with the earlier solutions a typical effect was only obtained if 0·05 to 0·1 cc. of the undiluted solution was injected. The well-tolerated dose must, therefore, be ascertained in the case of each solution injected.

It was found in mice so prepared that the red blood corpuscles of a chicken circulate in the blood for 24 to 36 hours, although in normal mice they have all disappeared in from 2 to 3 hours. The same holds true when *Sp. gallinarum* is injected. Not only the phagocytosis of trypanosomes, but the formation of trypanosomal antibodies is practically entirely excluded by this combined blockade. According to previous researches of the authors, the trypanocidal antibodies of the mouse are almost entirely formed in the spleen. This is proved by the facts that (*a*) infected splenectomized mice, which had been cured, exhibited no immunity against a second infection; and that (*b*) the trypanosomes appearing in the blood after the incomplete cure of splenectomized mice are sensitive to serum. Formation of serum-fast strains does not take place.

The authors' experiments indicate that humoral immunity plays an important part in the mechanism of cure, the specific antibodies killing off these trypanosomes which have escaped the chemotherapeutic shock. It follows from this that a definite cure may result even though the chemotherapeutic agent does not directly destroy all the trypanosomes in the body. The definite sterilization of the organism by minimal sterilizing doses of a drug is due in part to the intervention of immune bodies; and this accounts for the fact that when minimal doses of a drug are used splenectomy definitely interferes with the therapeutic effect. In such cases there is an early reappearance of the trypanosomes in the blood, even when the drug is given in doses which suffice to sterilize the normal animal.

The authors record some interesting observations on the mechanism of the action of germanin. They claim that the phagocytes of the reticulo-endothelial system play an important part in the curative mechanism, and that germanin exerts an opsonin-like action on the trypanosomes. As, however, after splenectomy and injection of the electro-colloidal copper solution, the natural mechanisms of defence are entirely excluded, owing to the poisonous effect of the colloidal copper solution on the reticulo-endothelial system, the phagocytosis of trypanosomes is abolished and trypanosomal immune bodies are produced only in traces. *W. Y.*

WALLACE (J. M.). **A Note on an Indirect Method of demonstrating Drug Resistance in Trypanosomes,** *in vivo.—Trans. Roy. Soc. Trop. Med. & Hyg.* 1934. Nov. 27. Vol. 28. No. 3. pp. 347–348.

The author points out that the disadvantage of the usual method of testing *in vivo* the resistance to arsenic of a strain of trypanosomes is the impossibility of giving a higher dose of the drug than is normally toxic to the host animal. In the experiments described in this paper guineapigs were treated with gradually increasing doses of tryponarsyl until they could withstand a dose which would be lethal if given as an initial dose. Twenty-four hours after the final dose of the drug "when the drug has had time to be absorbed from the peritoneal cavity," the animal is inoculated with the trypanosome believed to be resistant. The experiment, which is described in detail, was performed with the normal and resistant strains of *T. rhodesiense* sent to Uganda by the

reviewer. Wallace states that these experiments do not expose the trypanosomes to such a high concentration of drug as is possible *in vitro*, but that they enable the *in vivo* test to be extended, and they confirm existing views by indicating that drug-resistance is relative. [In the reviewer's opinion it will be necessary to know a good deal more about the fate, rate of excretion, etc., of a drug after its injection into the peritoneal cavity, before we are even justified in saying that this method of experimentation allows the *in vivo* test to be extended.]

W. Y.

i. CULBERTSON (James T.) & STRONG (Paul S.). **The Trypanocidal Action of Normal Human Serum. The Nature of the Substance Responsible for the Trypanocidal Effect and its Relationship with the Bactericidal Activity of Normal Human Serum.**—*Amer. Jl. Hyg.* 1935. Jan. Vol. 21. No. 1. pp. 1–17. [19 refs.]

ii. HANDLER (Bernard J.). **Studies on the Trypanocidal Power of Normal Human Serum.**—*Ibid.* pp. 18–26. [14 refs.]

i. The authors have studied the nature of the substance responsible for the trypanocidal action of normal human serum, and particularly its relationship to alexin, firstly as regards the effect of heat upon each, secondly as regards their respective filtrability, and thirdly as regards their fixability by trypanosomes and other substances.

It was found that heating normal human serum sufficiently to inactivate alexin does not destroy the trypanocidal property, although this may be slightly reduced in potency. A serum of which the alexin is inactivated by heating is not re-activated in trypanocidal power by the addition of fresh guineapig serum potent in alexin. Repeated filtration of normal human serum through a Berkefeld filter enabled the trypanocidal substance to be separated from alexin, the latter being held up much more readily than the former. From various experiments it is concluded that the trypanocidal power of normal human serum is in its action independent of the several known components of alexin.

The trypanocidal property can be inactivated or absorbed from normal human serum by trypanosomes and certain bacteria, but not by charcoal or kaolin. Carmine inactivates both the trypanocidal and bactericidal substances in normal human serum; and experiments indicated that there is some degree of correlation between the bacteriocidal titre and the trypanocidal titre of normal human serum. The general conclusion is that the agent responsible for the trypanocidal activity of normal human serum is a relatively non-specific antibody, perhaps similar to the substance responsible for bactericidal activity.

ii. The work described in this paper was devised with the object of reinvestigating the observations upon which ROSENTHAL and FREUND described the "exhaustion phenomenon" [this *Bulletin*, Vol. 20, p. 700; Vol. 27, p. 238]. This phenomenon is broadly to the effect that repeated injections of human serum into mice rendered a subsequent therapeutic inoculation of the same agent ineffectual. ROSENTHAL and FREUND interpreted their experiments as indicating that some activating substance in the mouse had been exhausted, and hence that human serum is not trypanocidal, but trypanocidogenic.

The work of the reviewer and his colleagues had, however, shown that normal human serum exerted a direct lytic action on trypanosomes

in vitro. It was therefore with the object of clearing up these discrepancies that Handler undertook his present work. Experiments showed that human serum heated to 60°C. for one hour is just as effective as unheated serum in evoking the " exhaustion phenomenon " of ROSENTHAL and FREUND ; this observation consequently failed to support the hypothesis advanced by these authors. Further experiment showed that the trypanocidal agent in normal human serum is capable of producing an equivalent antibody. When sufficient antiserum prepared against unheated human serum is added to normal human serum *in vitro*, neither the supernatant fluid nor the precipitate display any trypanocidal activity. But when antiserum prepared against heated human serum is used under similar conditions, the trypanocidal power of the supernatant fluid is not diminished. Similarly, the antagonistic effect *in vivo* could be elicited only with antiserum obtained by immunization with unheated human serum.

W. Y.

FUJIBAYASHI (Michizo). Studien ueber die trypanozide Kraft des Menschenserums. I. Ueber die trypanozide Kraft des Menschenserums und anderer Körpersäfte bei verschiedenen physiologischen und pathologischen Zuständen. II. Zusammenhang zwischen der trypanoziden Kraft des Menschenserums und der Blutplättchen. [**I. The Trypanocidal Power of Human Serum and Other Body Fluids in Various Physiological and Pathological Conditions. II. The Relationship between the Trypanocidal Power of Human Serum and the Platelets.**]—*Fukuoka Acta Med.* (*Fukuoka-Ikwad-aigaku-Zasshi*). 1934. Oct. Vol. 27. No. 10. [In Japanese pp. 2279–2308. [34 refs.] ; pp. 2309–2364. [38 refs.] German summaries pp. 121–122 ; 122–123.]

I. The trypanocidal power of human serum is greatest in adult age ; it may be increased or decreased by exercise. In cirrhosis of the liver and in cases of obstructive jaundice the trypanocidal power is either lost or at least decreased. It is also decreased in such diseases as Banti's, aplastic anaemia, secondary anaemia, haemophilia, myelogenous leukaemia and paroxysmal haemoglobinuria ; but in typhoid, phthisis and acute and chronic infectious diseases it is almost normal. The author also examined the trypanocidal power of organ extracts and of the extracts which had been concentrated to $\frac{1}{10}$ of their original volumes by means of a vacuum. In cirrhosis of the liver, neither the original extract nor the concentrated form exhibited trypanocidal power. The cerebrospinal fluid of various patients showed no trypanocidal action even when concentrated.

II. In adult life and in old age there seems to be no relationship between trypanocidal power and the number of platelets, but in adolescence there is a tendency towards increase in platelets if the trypanocidal power is increased. In cirrhosis of the liver, both trypanocidal power and the number of platelets are decreased. The author tested in *in vitro* and in *in vivo* experiments the trypanocidal power of blood platelet extracts prepared in various ways ; in 6 of 11 *in vitro* experiments and in 5 of 17 *in vivo* experiments a trypanocidal power was demonstrated. Finally, experiments were undertaken to ascertain whether the serum of rabbits immunized against normal human serum inhibited the trypanocidal power of human serum ; the results were negative, but the serum of rabbits immunized against blood platelet

extract did inhibit the trypanocidal action of normal human serum. As the result of this work the author believes that the platelets play some part in the formation of the trypanocidal substance. *W. Y.*

POINDEXTER (Hildrus A.). **A Thermoprecipitation Reaction in *Trypanosoma equiperdum* Infection in Laboratory Animals.**—*Jl. Experim. Med.* 1934. Nov. 1. Vol. 60. No. 5. pp. 575–579. [11 refs.]

The work described in this paper was undertaken in order to ascertain whether *T. equiperdum* possesses a thermoprecipitinogen. Extracts of various organs and tissues of rats, guineapigs, and rabbits infected with *T. equiperdum* were tested by means of the precipitation reaction for the presence of a substance which reacts with the serum of recovered animals.

The extracts were prepared by cutting the organs into small pieces and triturating them in the presence of talc. To the triturate was added five parts of normal saline or water for each gram of the original organ. The suspension was then boiled for 5 minutes and allowed to cool. The liquid was separated by centrifugation. The extracts were generally prepared and used on the same day; 0·5 cc. of the clear extract was layered upon an equal quantity of immune serum in a small agglutination tube. The results were read after 30 minutes at room or body temperature, and again after 18 hours in the ice box. Experience showed that the most strongly positive reactions were obtained after a period of 18 hours in the ice box. As a result of this work, it was found that extracts of the spleen of rats, guineapigs and rabbits infected with *T. equiperdum* contained a thermoprecipitinogenic substance; this substance was not found within the body of the trypanosome itself. Antibodies to it were present in the serum of infected animals. Whilst the antibody seemed to be relatively less in the serum of rats than in other animals, the thermoprecipitinogenic power of extracts of the spleen of infected rats was equal to that of similar extracts of other animals. *W. Y.*

KUNERT (H.) & KRAUSE (M.). Nachtrag zur Arbeit: Findet in *Glossina morsitans* eine zyklische Entwicklung von *Trypanosoma evansi* statt? [**Additional Note to the Authors' Paper on the Question of Cyclical Development of *T. evansi* in *Glossina morsitans*.**]—*Arch. f. Schiffs- u. Trop.-Hyg.* 1934. Dec. Vol. 38. No. 12. p. 534.

In answer to various inquiries the authors give information regarding the age of the strain of *T. evansi* used in their earlier experiments on cyclical development [this *Bulletin*, Vol. 31, p. 597]. It was isolated from a camel about the middle of October, 1932, maintained in mice until the end of October and thereafter in guineapigs. At the date of the experiments in question (1933) the strain was therefore 8 months old. *W. Y.*

PERLA (David). **The Protective Action of Copper against *Trypanosoma equiperdum* Infection in Albino Rats.**—*Jl. Experim. Med.* 1934. Nov. 1. Vol. 60. No. 5. pp. 541–546.

In previous work [*ante*, p. 42] it had been shown that the addition to an adequate diet of small quantities of copper or iron, or both, during

a period of 10 days prior to infection with *T. lewisi* raised the natural resistance of the rat to the disease. In the present communication the effect of additions of copper salts to the diet on a subsequently induced infection with *T. equiperdum* was determined. It was found that copper, in amounts equivalent to 0·2 mgm. of elemental copper per rat per day, during a period of 10 days prior to infection with small numbers of trypanosomes, increased the natural resistance of the rat to the infection. The infection was aborted in all instances when the number of trypanosomes injected was 2,000, and in 75 per cent. of cases when the rats were injected with 10,000 trypanosomes. *W. Y.*

CORSON (J. F.). **The Action of "Bayer 205" on** *Trypanosoma rhodesiense* **in White Rats infected by Tsetse-Flies.**—*Ann. Trop. Med. & Parasit.* 1934. Dec. 20. Vol. 28. No. 4. pp. 535–547. [22 refs.]

After giving a brief summary of previous experimental work on the prophylactic action of Bayer 205, Corson records certain observations made by himself. These, he states, are chiefly interesting because a recent strain of *T. rhodesiense* was used, transmitted by isolated infective laboratory-bred *G. morsitans*. The results are in general agreement with those of earlier work with old laboratory strains of trypanosomes, mechanically transmitted.

Details of the experiments are given in a series of tables. It was found that a dose of 0·015 gm. per kilo. of body weight did not protect white rats for 21 days against the bites of isolated tsetse flies infected with a recent strain of *T. rhodesiense*; and that a dose of 0·03 gm. per kilo. failed to protect for 40 days. In further experiments it was found that the restraining action of Bayer 205 on the development of trypanosomes was not seen in rats subinoculated from rats which had relapsed after treatment with this drug, nor in rats bitten by tsetses infected from treated rats during a relapse. *W. Y.*

DECOURSEY (Elbert). **The First Fatal Case of Chagas' Disease observed on the Isthmus of Panama.**—*Amer. Jl. Trop. Med.* 1935. Jan. Vol. 15. No. 1. pp. 33–40. With 3 figs.

This paper describes the findings at the autopsy on a black baby 3½ months old, which died at Land Lease in the Canal Zone. The history of the case is that the child became very weak after the first month; about 5 days before death there was fever, swelling of the face and extremities, and severe dyspnoea. On the last two days of life there was excessive vomiting and anuria.

The outstanding lesions were in the heart and in the brain. The entire myocardium contained an abundance of parasites and inflammatory cells. The lesions of the brain were quite different in that they appeared as inflammatory foci, which were fairly numerous, while the parasites were rare. The thyroid gland was unaffected, as were also the skeletal and unstriated muscles. *W. Y.*

DIAS (Emmanuel). Persistance de l'infection par le *Schizotrypanum cruzi* chez l'homme. [**Duration of the Infection of** *T. cruzi* **in Man.**]—*C. R. Soc. Biol.* 1934. Vol. 117. No. 31. pp. 506–507.

Details are given of a case in the blood of which *T. cruzi* was found, both by the method of inoculation of guineapigs and by the

xenodiagnostic method of BRUMPT, after the patient had been in the Oswaldo Cruz hospital at Rio de Janeiro for 12 years.

When she was admitted to hospital in 1922 she had a goitre with cretinism and pronounced mental changes. As she came from a *T. cruzi* infested district it was considered that she was probably suffering from Chagas' disease, an opinion which was confirmed by the fact that the serum gave a strongly positive Machado reaction. Many attempts to infect guineapigs by inoculation with the patient's blood failed, and it was not until 1934 that the blood was proved to contain trypanosomes. The author states that this case shows that *T. cruzi* can exist in the human body for many years. *W. Y.*

CHAGAS (E.). Infection expérimentale de l'homme par le *Trypanosoma cruzi.* [**Experimental Infection of Man with** *T. cruzi.*]—*C. R. Soc. Biol.* 1934. Vol. 117. No. 30. pp. 390–392. With 3 figs.

Further information is given regarding the patient experimentally infected with *T. cruzi* [*ante*, p. 37]. The patient died of cancer just over 6 months after inoculation with the trypanosome. Although the blood remained constantly infective for guineapigs, the patient presented few signs of the trypanosomal infection apart from certain electrocardiographic changes. At the post-mortem only the heart showed pathological changes due to the infection ; and it was only in this organ that leishmania-forms were found. The predilection of *T. cruzi* for the myocardium is thus clearly indicated. *W. Y.*

CHAGAS (Evandro). L'infection expérimentale chez l'homme par le *Schizotrypanum cruzi.* [**Experimental Infection of Men with** *T. cruzi.*]—*C. R. Soc. Biol.* 1935. Vol. 118. No. 3. pp. 290–292.

In these experiments three patients suffering from incurable malignant disease were employed. The conclusion was reached that *T. cruzi* cannot pass through the unbroken skin, but that it readily traverses the intact conjunctiva.

The first experiment consisted in ascertaining whether *T. cruzi* from the faeces of a Triatoma infected from a human case would pass through the unbroken skin ; and the second experiment was similar, except that the Triatoma was very heavily infected with a virulent strain of *T. cruzi* from an armadillo. Both were negative. In the third experiment material from the same source as used in the second experiment was deposited on the intact conjunctiva of a patient. Twelve days later there was a febrile disturbance accompanied by oedema of the eye. A guineapig inoculated with the blood two days later became infected. *W. Y.*

WOOD (Fae Donat). **Experimental Studies on** *Trypanosoma cruzi* **in California.**—*Proc. Soc. Experim. Biol. & Med.* 1934. Oct. Vol. 32. No. 1. pp. 61–62.

Observations are described on *T. cruzi* isolated from *Triatoma protracta* in California. The faeces of 54 per cent. of Triatoma in San Diego County were infected, but nothing was found in the faeces of bugs collected in the vicinity of Berkeley and Los Angeles. Of 43 San Diego wood rats examined, one was found to be infected, thus incriminating

the animal as a reservoir host of *T. cruzi*. One hundred and thirty-four animals, belonging to 16 different species, were inoculated with the Californian strain of *T. cruzi*, and a list is given showing the number of each which became infected. Attempts made to intensify the infection by lowering the host's resistance by splenectomy, by injection of testicle extract, and by keeping the animals at a higher temperature, failed. Successive passages through different host species, covering a period of 103 days, indicated a stimulating effect upon the trypanosomes in that the incubation period progressively decreased from 35 to 20 days. *W. Y.*

WOOD (Fae Donat). **Natural and Experimental Infection of** *Triatoma protracta* **Uhler and Mammals in California with American Human Trypanosomiasis.**—*Amer. Jl. Trop. Med.* 1934. Nov. Vol. 14. No. 6. pp. 497–517. With 24 figs. on 3 plates. [12 refs.]

The author states that for a period of four years he has been engaged upon the study of the life history of a trypanosome described in 1916 by KOFOID and MCCULLOCH from the digestive tract of *Triatoma protracta* found in nests of the San Diego wood rat. An extensive field of laboratory investigations has indicated that this parasite is a relatively non-virulent strain of *T. cruzi*.

The author summarizes the results of his work as follows :—

" 1. The blood-sucking bug, *Triatoma protracta* Uhler, and the wood rat, *Neotoma fuscipes macrotis* Thomas are natural carriers of *Trypanosoma cruzi* Chagas in Southern California.

" 2. The following animals have been experimentally infected with this trypanosome : albino rats, albino mice, rhesus monkeys, a puppy, an opossum (*Didelphis virginiana virginiana* Kerr), 2 species of dusky-footed wood rats (*Neotoma fuscipes annectens* Elliot and *N. f. macrotis* Thomas), and 5 species of white-footed mice (*Peromyscus eremicus fraterculus* [Miller], *P. californicus insignis* Rhoads, *P. californicus californicus* [Gambel], *P. maniculatus gambeli* [Baird], *P. truei gilberti* [Allen]).

" 3. The San Diego desert and southern parasitic mice and the Virginia opossum have all been found in wood rat nests in the infected locality, so it is possible that they, too, are carriers.

" 4. Leishmania bodies were seen in bone marrow, cardiac and voluntary muscle of infected animals.

" 5. Lesions composed of infiltration lymphocytes, monocytes, and plasma cells have been found in cardiac and voluntary muscles, cerebrum, and meninges.

" 6. Animals infected by this strain take light infections, showing few parasites or lesions and usually no symptoms.

" 7. Neither splenectomy, injection of testicle extract, nor increased temperature have any intensifying effect upon the infection.

" 8. Varying the host species gave progressively shorter incubation periods, indicating a stimulating effect upon the parasite.

" 9. One out of five attempts to reinfect animals succeeded, indicating a partial immunity.

" 10. This trypanosome has been cultured on semi-solid blood agar, the culture forms being comparable to the insect phase." *W. Y.*

DIAS (Emmanuel). Le xénodiagnostic appliqué à la trypanosomiase américaine. [**Xenodiagnosis in American Trypanosomiasis.**]—*C. R. Soc. Biol.* 1935. Vol. 118. No. 3. pp. 287–289.

The author examined the blood (fresh and stained preparations) of over 100 inhabitants of Lassance, in the State of Minas Geraes, for

T. cruzi, and in a proportion of these cases the parasite was also sought by the xenodiagnostic method of Brumpt and by blood culture.

Most of the people examined lived in primitive huts of the kind favoured by Triatoma, which abounded. In all, the blood of 113 persons was examined microscopically; the results were negative: from 16 of these blood cultures were made, and here again the results were negative. The xenodiagnostic method was applied in 30 of the cases in whom blood examination had proved negative. For this work 3rd stage larvae and nymphs of laboratory-bred *Triatoma megista* were used. These insects had been fed only on clean guineapigs before the experiment, and flagellates had never been found in such laboratory-bred Triatoma.

In all, 147 Triatoma fed on 38 persons, the number feeding on each varying from two to six. Between 41 and 51 days later the insects were dissected and five were found to be infected with *T. cruzi*. By this means two of the 38 persons were therefore discovered to be infected. Details of these cases are given. *W. Y.*

VILLELA (E.) & DIAS (Emmanuel). Sur la formation d'ulcérations chez les animaux infectés par le *Schizotrypanum cruzi*. [**Ulcerations in Animals infected with** *T. cruzi*.]—*C. R. Soc. Biol.* 1934. Vol. 117. No. 30. pp. 394–396.

A description is given of ulcerative lesions occurring spontaneously in dogs experimentally infected with *T. cruzi*. The ulcers may appear during the acute stage of the disease or later.

The lesions have the appearance of necrotic ulcers, circular in shape and with sharp margins; they are painful, develop rapidly, and penetrate deeply, sometimes reaching the bones or joints. Sections of deep lesions showed inflammatory foci, sometimes around the sebaceous follicles and at other times in the papillae of the dermis. The foci consist of endothelial cells or plasma cells or both; they are never formed by polymorphonuclear cells. Parasites were not found in the foci studied. In the sections of the open lesions (ulcers of the mouth, vagina and skin), foci containing parasites in greater or less numbers were found. These findings reminded Villela of the necrotic ulcerations he described in a human case of the disease in 1923; similar lesions have also been recorded in the naturally infected armadillo. These facts indicated once again the biological affinities of *T. cruzi* with the genus *Leishmania*. *W. Y.*

VILLELA (Eurico) & DIAS (Emmanuel). Localisation des formes de multiplication du *Schizotrypanum cruzi* dans la peau et dans les muqueuses de chiens expérimentalement infectés. Parasitisme de la cellule épithéliale de l'épiderme. [**Localization of Multiplication Forms of** *T. cruzi* **in the Skin and Mucous Membranes of Experimentally Infected Dogs.**]—*C. R. Soc. Biol.* 1934. Vol. 117. No. 31. pp. 501–504. With 3 figs.

Further details are given of the localization of the parasites in the cellular elements of the skin and intestinal mucous membrane of dogs infected with an armadillo strain of *T. cruzi*.

As mentioned in their previous paper, this strain frequently produced cutaneous ulcerations. In certain cases sections of the cutaneous ulcers showed an intense parasitism of the lesions. As usual the leishmania

forms were found in great number in the histiocytes of the cutaneous tissue. The elements situated in the vicinity of the hair follicles and sebaceous glands were chiefly involved; they presented at the periphery a more or less intense reaction characterized by an infiltration of mononuclear cells, plasma cells, and in the ulcerated regions by an agglomeration of polymorphonuclear cells and a deposit of fibrin and the formation of a micro-abscess. ADLER and others have drawn attention to the similar localization of *Leishmania canis* in the vicinity of hair follicles and sebaceous glands.

The most remarkable fact observed by the authors was the presence of parasitized cells in the epidermis. Parasites were also found in the gastro-intestinal submucosa. In the stomach of one dog a large focus of infected cells was found which extended up to the muscularis mucosae close to the epithelium. *W. Y.*

TANGANYIKA TERRITORY. **A Further Account of the Anti-Tsetse Campaign in Tanganyika Territory by Officers of the Tsetse Research Department.**—Reprinted from *Tanganyika Standard* between Oct. 17, 1933 and May 1, 1934. 32 pp.

To the intelligent layman wishing to know what the Tsetse Research Department in Tanganyika Territory has done and is doing, this interesting pamphlet should convey much useful and reassuring information. The scope of the publication is sufficiently indicated by the following chapter-headings: "Driving the Tsetse by Fire"; "Anti-Tsetse Clearings by Tribal Labour"; "Advances of Tsetse Fly"; "The Provision of Water in Reclaimed Country"; "Wresting Large Areas of Grazing from the Tsetse"; "Experiments in the Elimination of the Tsetse Fly by Means of 'Faunal' Control"; "Elimination of the Tsetse Fly by Means of 'Floral' Control"; and "A Concluding Survey of our Tsetse-Flies and of the Present Prospects of Controlling Them."

Of the seven species of tsetse in Tanganyika Territory, by far the most important are *Glossina morsitans*, *G. swynnertoni* and *G. pallidipes*, and the majority of the fly-belts are either "spreading or nearly stationary"; spreading is believed to be reconquest of territory occupied by the insect perhaps before the appearance of man. In Shinyanga, as a result of measures carried out under the auspices of the Tsetse Research Department, the fly has been not only stopped but driven back, and 30,000 people, previously forced to abandon their homes, are now gradually returning.

Facts and arguments on both sides in the perennial "tsetse-fly—big game" controversy are impartially presented, and, on behalf of the game, it is concluded that "if the game is so reduced that the fly-population [*G. morsitans* group] becomes hungry, wide spread may be expected." The special merits of "floral" or "vegetational" control, *i.e.*, the *alteration* instead of destruction of the plant-communities which shelter the fly, are explained, and this method of attack is considered to be "perhaps the most promising of all." *E. E. Austen.*

LEWIS (E. Aneurin). **Tsetse-Flies in the Masai Reserve, Kenya Colony.** —*Bull. Entom. Res.* 1934. Dec. Vol. 25. Pt. 4. pp. 439–455. With 1 folding map.

The Masai Reserve lies on the Tanganyika border and comprises some 10,000,000 acres, of which about 800,000, though of pasturage,

are infested by tsetse-flies and therefore useless. Extending " from about 34° 37′ to 38° 15′ E. Long. ; and from about 0° 34′ to 3° 10′ S. Lat.", the Reserve, described in some detail in the first part of this paper, includes a wide variety of country, altitude and vegetation. Within the Reserve six species of *Glossina* (*G. brevipalpis*, *G. fuscipleuris*, *G. longipennis*, *G. pallidipes*, *G. palpalis* and *G. swynnertoni*) are found, and notes are given on the local occurrence of each of these. The presence of *G. swynnertoni*, a vector of human as well as animal trypanosomiasis, hitherto believed to be confined to the Mwanza district of Tanganyika, may well prove to be a matter of some importance, since Osero, a district of about 700 square miles forming the south-western region of the Reserve, is believed to be " completely infested " with this fly, as well as with *G. pallidipes*.

It would seem that, since the institution of the present Masai Reserve, the fly-belts within its confines have extended their boundaries and increased in number ; and in the case of *G. swynnertoni* available evidence tends to show that there has been an actual invasion from Tanganyika Territory. The readiness with which *G. swynnertoni* attacks man in the presence of cattle, but where game is scarce, has previously been recorded by SWYNNERTON ; in the Masai Reserve the present author found that :—" In the presence of an abundance of game and in the absence of cattle . . . *G. swynnertoni* very readily approached man and was attracted to moving vehicles such as cars and lorries."

While tsetse-fly infestation of the Masai Reserve is serious, the customs and traditions of the people in relation to manual labour are likely to militate against the possibility of reclaiming the known infested areas. *E. E. A.*

JACKSON (C. H. N.). **A Note on the Concentrations of Tsetse-Flies.**—*Bull. Entom. Res.* 1934. Dec. Vol. 25. Pt. 4. pp. 457–458.

During the dry season in Tanganyika *Glossina morsitans* increases in numbers in actual vleis or drainage valleys, " as distinct from the bordering woodland." Observations by BURTT at a waterhole in a drainage valley or narrow vlei, after the beginning of August, support the author's contention that " the increase of fly in the vlei in the hot, dry months is due, not to a search for better shade conditions, but to the fact that the vlei is a feeding-ground, and that the fly must visit it more frequently at the season when . . . the onset of hunger is hastened by hot, dry conditions." *E. E. A.*

SWYNNERTON (C. F. M.). **Protection of Vegetation against Grass-Fires as a Possible Solution for Some Tsetse Problems.**—*Bull. Entom. Res.* 1934. Sept. Vo. 25. Pt. 3. pp. 415–430. With 1 plan, 1 folding chart, & 12 figs. on 4 plates. [12 refs.]

In Tanganyika Territory, to which this paper refers, it was found in 1925 " that even the relatively low deciduous thicket of the Central Province . . . tended strongly to exclude *Glossina morsitans*." It was thereupon decided " to test the possibility of reproducing these inimical conditions (*a*) by advancing the natural vegetational succession by not burning the grass, (*b*) by cheap or remunerative planting." The results of experiments on *G. swynnertoni* and *G. pallidipes* at Shinyanga in pursuance of this policy are here described. Although the cessation of annual burning of vegetation has had little effect upon the game,

certain sections of road on which tsetse were formerly "a serious nuisance" are, now that they are protected by thicket barriers, relatively free from fly. Barriers of deciduous thicket, impassable to fly in the wet season, may be so contrived as to restrict tsetse to areas insufficient for their needs. Yet the effects of not burning will differ with the species of tsetse concerned. "*G. morsitans* can exist under more humid conditions than does *G. swynnertoni*." *G. brevipalpis*—not perhaps of great practical importance—is, owing to the nature of its normal habitat, more likely to be assisted than otherwise by the cessation of annual fires. It remains to be proved whether the more dangerous *G. pallidipes*, which in Tanganyika occurs throughout more than half the area occupied by *G. morsitans* and *G. swynnertoni*, will be helped or hindered.

In selected sites, carefully controlled experiments are to be made in connection with game, by means of which it is hoped to discover whether the fly is specially dependent upon particular species; whether the fly can be abolished by "anything short of the complete extermination or expulsion of animals"; and "whether such extermination (locally) is practicable by such means as are cheaply available to a government."

Pending the final results of investigations as yet unfinished, the author is inclined "to regard the release of the vegetational succession by the prevention of grass-burning as likely to be useful as a *basic* measure," to be supplemented or replaced by others as and where required. *E. E. A.*

HENRARD (C.). Quelques essais de capture de *Glossina palpalis* au moyen de divers types de piège Harris près du Stanleypool. [**Trials of Various Types of Harris Trap for the Capture of** *G. palpalis* **near Stanley Pool.**]—*Ann. Soc. Belge de Méd. Trop* 1934. Sept. 30 Vol. 14. No. 3. pp. 263–276. With 2 figs.

The tests described were carried out between October 1933 and February 1934, on an island in the Congo west of Leopoldville, whereon wild animals were present in limited numbers, while crocodiles were numerous in the vicinity. The greatest average density of *G. palpalis* where the fly was most numerous was from 10 to 15 per fly-boy hour. Four traps were used, and of these the most efficient was one capable of being taken to pieces, and when in use suspended from a branch. While the average number of tsetse caught per trap during a month of fine weather was 2,000, the monthly figure for the whole period was only 850.

Traps were found to yield the best results when men or animals were present or passed by [*cf.* SWYNNERTON on the value of animal scent, this *Bulletin*, Vol. 30. p. 618]. The author's final conclusion is that Harris traps, appropriately sited, can usefully be employed for the local protection of human beings and domestic animals. *E. E. A.*

BUXTON (P. A.) & LEWIS (D. J.). **Climate and Tsetse Flies: Laboratory Studies upon** *Glossina submorsitans* **and** *tachinoides*.—Reprinted from *Phil. Trans. Roy. Soc. London.* Ser. B. 1934. Dec. 14. Vol. 224. No. 512. pp. 175–240. With 14 text figs. & 5 figs. on 2 plates. [38 refs.]

Previous workers have studied the effects of climate upon wild populations of Glossina, and have obtained valuable results. The

authors of this paper have worked in the laboratory, and have attempted to analyse the effects of controlled conditions of temperature and humidity. Many suggestive results were obtained, which should greatly encourage further work of this sort. The work was done in the laboratory of the Tsetse Investigation at Gadau in Northern Nigeria.

The upper and lower limits of temperature at which the adult flies could survive were found, and the effects of humidity on the thermal death points were investigated. The time which adults could survive each controlled condition was determined, in some experiments using starved individuals and in others giving the flies opportunities of feeding daily. The rate of reproduction under these conditions was also studied. The rate of loss of water and of fat metabolism was found. Experiments were also made with puparia. Finally, continuous records of temperature and humidity were made both in an open clearing and in a dense thicket, in dry and wet seasons.

While the effects of temperature appear to be simple—there is a fairly narrow zone within which the fly can live satisfactorily—the effects of humidity are more complex. With a temperature of 30°C., a relative humidity of about 44 per cent. appears to be near the optimum at which the flies live longer and breed more rapidly than in drier or in moister air. A relative humidity of only 65 per cent. was unfavourable, and in moister air the flies died off very rapidly, and fed with reluctance. The reason why high humidities are unfavourable is still obscure.

At temperatures above 40°C. the adult flies survive better in dry air than in moist. They also metabolized fat most rapidly in dry air, presumably to produce metabolic water to compensate for excessive evaporation.

The puparium was shown to have an optimum humidity near saturation, and it is suggested that the air in the spaces in apparently powder-dry soil may be much moister than is usually imagined, even when the general atmosphere is very dry. Unfortunately the soil conditions were not investigated further.

The field meteorological results support the laboratory work most satisfactorily. When the humidity was high, the flies were scarce, and many workers have found that pregnancies are rare under these conditions.

Various practical measures of control, such as clearing of undergrowth, are discussed in the light of the experimental results.

K. Mellanby.

De la Camara (Pedro). Accion de algunos arsenicales organicos sobre la morfologia de los tripanosomas.—*Medicina Países Cálidos.* Madrid. 1934. Oct. Vol. 7. No. 10. pp. 460–469. With 2 figs. & 1 coloured plate. English summary (8 lines).

Geoghegan (Arnoldo J.). Nuevo caso de tripanosomosis humana en la ciudad de Catamarca.—*Rev. Inst. Bacteriológ.* Buenos Aires. 1934. Mar. Vol. 6. No. 2. pp. 212–215. With 2 figs.

Geoghegan (Arnoldo J.). Nuevo caso de tripanosomosis humana en Huillapima (Catamarca).—*Rev. Inst. Bacteriológ.* Buenos Aires. 1934. Mar. Vol. 6. No. 2. pp. 216–219. With 3 figs.

MAZZA (Salvador); CORNEJO (Andrés). Casos agudos benignos de enfermedad de Chagas comprobados en la Provincia de Jujuy.—*Rev. Med.-Cirurg. do Brasil.* 1934. Sept.–Oct. Vol. 42. Nos. 9–10. pp. 308–316.

ROMAÑA (Cecilio). Novas investigações sobre a molestia de Chagas na Republica Argentina.—*Rev. Med.-Cirurg. do Brasil.* 1934. Sept.–Oct. Vol. 42. Nos. 9–10. pp. 298–307.

TORREALBA (J. F.). El primer caso de tripanosomosis americana diagnosticado en el estado Guárico por el exámen directo de la sangre.—*Gac. Med. de Caracas.* 1934. Sept. 30. Vol. 41. No. 18. pp. 275–279. [16 refs.]

UNIVERSIDAD BUENOS AIRES MISIÓN DE ESTUDIOS DE PATALOGÍA REGIONAL ARGENTINA JUJUY. 1934. Publicación No. 18. 32 pp. With 25 figs. Investigaciones sobre la enfermedad de Chagas. I. Casos crónicos de enfermedad de Chagas determinados en Jujuy [MAZZA (Salvador)]. II. Casos crónicos de enfermedad de Chagas, demostrados en Salta [MAZZA (Salvador) & CORNEJO (Andres)].

VENOMOUS SNAKES AND SNAKE VENOMS. II.*

Not many new data have been published in regard to geographical distribution of the Ophidia. PHISALIX and HOUDEMER[1] report on the venomous snakes of Indo-China. Of the proteroglyphous colubrids both the Hydrophinae and Elapinae are represented. The sea snakes observed on the Indo-China coast include species of the genera *Platurus, Hydrophis, Distira, Hydrus, Enhydris* and *Enhydrina,* the venom of the latter being ten times as toxic as cobra venom. The elapines include four genera, *Callophis, Doliophis, Bungarus* and *Naja*; the most common species are *Bungarus fasciatus, Naja tripudians* and *Naja bungarus.* The crotaline vipers are represented by species of the genera *Ancistron* and *Lachesis.*

I. *Ophidian Dentition and Evolution of the Poison Fang.*

SMITH[2] introducing this subject at the Royal Society of Medicine pointed out that snakes had originally been derived from lizards or some lizard-like creature, and that most of the primitive snakes still showed some evidence of their four-footed ancestry, notably by the presence of a pelvis and vestiges of hind limbs. From one of these primitive groups the family Colubridae had been formed, and it was from the Colubridae that the poisonous snake had been derived. The Colubridae were a large family and capable of division in terms of their dentition into the Aglypha with solid non-grooved teeth, the Ophisthoglypha which have grooved teeth or fangs at the back of the mouth, and the Proteroglypha, which have tubular or canalized anteriorly-situated fangs. The Ophisthoglypha had been derived from the Aglypha and both have in their turn given rise to the Proteroglypha. The venomous snakes were late arrivals upon the earth and represented a high degree of specialization in which the evolution of the poison fang was secondary to the salivary venom gland. Actually the back-fanged snakes were only rarely venomous to man, the vast majority being quite harmless. The front-fanged snakes on the other hand—the Proteroglypha—were highly toxic; they could be divided into two groups having divergent lines of evolution—the Elapidae and the Viperidae.

In the Elapidae the poison fangs were comparatively short and the maxillary bone long, having behind one to eighteen smaller grooved teeth; not infrequently the teeth upon the palatine and dentary bones were more or less distinctly grooved, but never tubular. The normal position of the elapine fang was almost vertical in the mouth, approximately at right angles to the maxillary bone.

In the Viperidae the maxillary bone, which was very short and bore the fangs only, was moveably attached to the prefrontal and the ectopterygoid so that during striking the bone and the fang upon it could be erected. The resting position of the viperine fang was almost horizontal in the mouth, and the ability to fold these greatly elongated fangs back had become imperative for the preservation of the species.

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*For the first of this series see Vol. 31, p. 99.

[1] PHISALIX & HOUDEMER (E.). Contribution à la faune venimeuse du Tonkin.—*Bull. Soc. Path. Exot.* 1934. Feb. 14. Vol. 27. No. 2. pp. 178–184.

[2] SMITH (Malcolm A.). The Classification of Snakes in Accordance with their Dentition and the Evolution of the Poison Fang.—*Proc. Roy. Soc. Med.* 1934. June. Vol. 27. No. 8. pp. 1081–1083 (Sec. Trop. Dis. & Parasit. pp. 43–45).

Nothing was known concerning the evolution of the poison fang in the elapine snakes ; no non-venomous snakes with grooved anterior teeth have been encountered, while the initial stages which have led to the formation of the elapine type of fang have not yet been found. The vipers, on the other hand, have obviously been derived from the Ophisthoglyphous snakes by the gradual movement forward and shortening of the maxillary bone ; as this bone became progressively shorter more and more teeth on its anterior part were lost until finally a stage was reached when only the posterior fangs remained ; these would now be located in the front of the mouth. One could arrange amongst the Ophisthoglypha a complete series showing this gradual shortening of the maxillary bone starting with *Oxybelis*, which has twenty or more teeth in front of the fangs, and culminating in *Miodon* which has only two. The ability to erect or depress the fangs became developed as the maxillary bone grew shorter and *Xenodon*, one of the Aglyphous snakes, actually could move its maxillary bone like the vipers. The power to erect in some degree the maxillary bone was widely spread among the Proteroglypha and in fact was only a further development of the power to move the jaw independently which all snakes possess.

II. *The Mechanism of Bite.*

FAIRLEY[3] at the same meeting dealt with methods of taking dental impressions of the bite and the significance of the " maxillary index " and the " quadrate index " which he had introduced for determining the biting efficiency of the Australian colubrids. In snake bite four distinct phases were recognized : (1) the strike ; (2) opening the mouth and elevation of the fangs ; (3) closing the mouth and the injection of venom ; (4) retraction of the fangs. In the Australian colubrids there was a wide range of variation in the mobility of the fangs, the degree of elevation from extreme retraction to maximal protraction varying from 10°–15° to 45°–50° in the different species studied. Each pterygo-palatine-transverse arch acted as a single entity, and when the protractor muscles of the palate drew the endo-pterygoid forward, they invariably brought with it the palatine bone and the ecto-pterygoid which impinged on the posterior arm of the maxilla, driving the maxilla forwards and upwards on the articulating surface of the prefrontal. This produced a variable degree of elevation and forward rotation of the fangs which were ankylosed to the inferior surface of the maxilla ; its extent could be judged by the angle formed at the ecto-pterygoid maxillary junction which, in the resting position, formed a straight line. Should this movement be doubted it could readily be demonstrated by pithing the snake, dissecting up the mucous membrane on the roof of the mouth and electrically stimulating the protractor and retractor muscles acting on the palatine arch ; alternatively, skulls could be prepared with the palatine arch in different positions. In these snakes the smaller the maxilla (*i.e.*, the greater the maxillary index), the greater the forward movement of the pterygo-palatine-transverse arch and the greater the degree of forward projection of the fangs. This mechanism differed from that of the vipers, in which the movement of the maxilla on the prefrontal was a true rotary one and not a forward and upward sliding movement as described above.

[3] FAIRLEY (N. Hamilton). Snake Bite : its Mechanism and Modern Treatment. —*Proc. Roy. Soc. Med.* 1934. June. Vol. 27. No. 8. pp. 1083–1091 (Sec. Trop. Dis. & Parasit. pp. 45–53). [23 refs.]

III. *Venom Yields.*

FREEMAN and KELLAWAY[4] report on the venom yields obtained by milking the common Australian snakes over a period of several years. For the tiger snake (*Notechis scutatus*) the average yield for 3,214 milkings equalled 0·0276 grams of dry venom, and for the black tiger snake (*Notechis scutatus* var. *Niger*) the average of 516 milkings was 0·07 grams. For the death adder (*Ancanthophis antarcticus*) 926 milkings from 360 snakes showed an average yield of 0·04 grams, while for the copperhead (*Denisonia superba*) the average of 1,940 milkings equalled 0·0215 grams. The latter species does not thrive well in captivity and the primary are considerably larger than the secondary yields: they were found to do better if milked at 6 instead of 3 weeks' interval. The average yield of 579 milkings from 170 black snakes (*Pseudechis porphyriacus*) was 0·03 grams: they seldom survived longer than 7 to 8 months. Fifty brown snakes (*Demansia textilis*) were under observation and these did least well in captivity; the average yield of 126 milkings was only 0·002 grams; this, however, does not represent the venom injected at a single bite in nature, as one brown snake measuring nearly 7 feet in length yielded 0·045 grams at a first bite, and an addition 0·0222 grams on milking.

IV. *Bacterial Flora of Snakes' Mouths.*

WILLIAMS, FREEMAN and KENNEDY[5] investigated this question in captive Australian snakes. They found that freshly caught snakes did not possess a very numerous oral bacterial flora, but in captivity the number of organisms present in their mouths multiplied enormously and included anaerobes, non-lactose-fermenters, coliform bacilli and staphylococci. Freshly collected venom contained many fewer organisms and about 30 per cent. of samples were sterile, those infected containing only one or two species of organism. Over 50 per cent. of the samples of adequately dried venom were sterile, the drying apparently killing off many of the non-lactose-fermenters. The ordinary methods of collection and handling of venom, though they excluded gross contamination by saliva, did not guarantee sterile dry venoms. Measures for the exclusion of laboratory contamination of the venom are described. "Cancre" in captive snakes was frequently found to be associated with a strain of *Proteus* or with a small Gram-negative cocco-bacillus.

V. *Some Rarer Australian Snakes and their Venoms.*

KELLAWAY[6, 7, 8] in a series of papers reports investigations on some of the rarer Australian snakes and their venoms, including *Denisonia*

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[4] FREEMAN (Mavis) & KELLAWAY (C. H.). The Venom Yields of Common Australian Poisonous Snakes in Captivity.—*Med. Jl. Australia.* 1934. Sept. 22. 21st Year. Vol. 2. No. 12. pp. 373–377.

[5] WILLIAMS (F. Eleanor), FREEMAN (Mavis) & KENNEDY (Eileen). The Bacterial Flora of the Mouths of Australian Venomous Snakes in Captivity.—*Med. Jl. Australia.* 1934. Aug. 11. 21st Year. Vol. 2. No. 6. pp. 190–193.

[6] KELLAWAY (C. H.). The Venom of the Ornamented Snake *Denisonia maculata.* —*Australian Jl. Experim. Biol. & Med. Sci.* 1934. June 16. Vol. 12. Pt. 2. pp. 47–54. With 2 figs.

[7] KELLAWAY (C. H.). The Venoms of Some of the Small and Rare Australian Venomous Snakes.—*Med. Jl. Australia.* 1934. July 21. 21st Year. Vol. 2. No. 3. pp. 74–78. With 1 fig.

[8] KELLAWAY (C. H.). The Venoms of the Broad-Headed Snake (*Hoplocephalus bungaroides*) and of the Yellow-Banded Snake (*Hoplocephalus stephensi*).—*Med. Jl. Australia.* 1934. Aug. 25. 21st Year. Vol. 2. No. 8. pp. 249–255. With 1 fig.

maculata var. *devisii* (South-Western Queensland). A bite in man by this snake was followed after an interval by sudden loss of consciousness, from which recovery occurred some few hours later with dramatic suddenness. A somewhat similar phenomenon was observed in rabbits injected intravenously with sub-lethal doses of venom. The venom itself appeared rather less potent than that of the copper-head both in respect of its haemolytic and neurotoxic properties, and had a strong paralysing action—which was spontaneously reversible—on the phrenic motor endings in the diaphragm.

Four other small species of Denisonia were studied, *D. daemelii* Günther, *D. flagellum* McCoy (the little whip snake), *D. suta* Peters and *D. coronoides* Günther (the white-lipped snake); their venom in all instances was similar to the common copper-head, *Denisonia superba*, but, unlike it, none was dangerous to man or large animals. The venom of *Demansia olivacea* Gray, apart from the possession of feeble thrombin, bore very little resemblance to the highly potent venom of *Demansia textilis*, while that of *Demansia psammophis* Schlegel was not highly toxic either. The venom of the *Furina annulata* Dumeril contained no coagulent principle and was not highly poisonous. None of these snakes was dangerous to man and their slender build went hand in hand with narrow heads, small venom glands and small venom yields, a correlation originally described by FAIRLEY and SPLATT when studying the larger and commoner Australian snakes.

Of the three species of the genus *Hoplocephalus*, no specimens of *H. bitorquatus* Jan were available; according to KINGHORN this snake is venomous, but not deadly. The broad-headed snake, *H. bungaroides* Boie, is an aggressive snake attaining 5 feet in length and must be regarded as definitely dangerous. Its broad head also suggests a large venom yield, though no information on this point is available. Its venom, judged by injection in three species of animals, is of about the same order of toxicity as that of the copper-head and resembles the tiger snake in possessing a powerful thrombin, only a feeble haemolytic action and inducing peripheral paralysis causing death by respiratory failure; its local action is more severe than any other of these venoms. As it is a rare snake KELLAWAY tested the neutralizing effects of tiger snake antivenenes on the venom of *H. bungaroides* and found very effective protection; its use is therefore advocated clinically.

A single specimen of the yellow-banded snake, *H. stephensi*, was studied in captivity for 3 months: its venom was found to resemble *H. bungaroides*, but the relatively small size of its venom yield makes it unlikely ever to prove fatal to man.

VI. *Toxicity of the Crotaline Venoms of Formosa.*

KYU[9], who previously has published data on the toxicity of the venom of *Trimeresurus gramineus* Shaw, and *T. mucrosquamatus* Cantor, now reports on that of *Ancistron acutus* Günther, for frogs, mice and rabbits. The potency of the venoms was in the above order and did not correspond to the human mortality figures from snake bite, which were *T. gramineus* (1 per cent.), *T. mucrosquamatus* (9 per cent.),

[9] KYU (Kenten). Toxikologische Untersuchungen ueber die Gifte der Crotalinae Formosa's. III. Mitteilung. Studien ueber das Gift von *Agkistrodon acutus*, Günther.—*Taiwan Igakkai Zasshi* (*Jl. Med. Assoc. Formosa*). 1933. Nov. Vol. 32. No. 11 (344). [In Japanese pp. 1500–1522. With 7 figs. German summary pp. 148–151.]

and *A. acutus* (23 per cent.). The anomaly is explained on the basis of the different amounts of venom secreted for the three species. SUZUKI, MATSUMOTO and SUGIO[10] determined the minimum lethal dose of the above Formosan venoms for the mongoose as well as for *Bungarus multicinctus* and *Naja naja atra*, and compared the relative resistance of the mongoose, rabbit and guineapig. The mongoose was far more resistant to the venoms of *Naja naja atra* and *Bungarus multicinctus* than to the others. The serum of the mongoose was found to neutralize *Naja* venom, but not that of the other snakes, and this power was considerably diminished by heating to 56°C. for 15 minutes and lost after 30 minutes at that temperature. The red corpuscles of the mongoose could also neutralize *Naja* venom, a power not possessed by the leucocytes.

VII. *Pharmacological and other Observations on Snake Venoms.*

VELLARD and VIANNA[11] group the actions of various venoms into (1) neurotropic including the curari and hypotensive effects; (2) action on phosphatids which play a part in haemolysis, coagulation and cytolysis; (3) action on proteids destroying fibrinogen and complement and causing local oedema, haemorrhages and gangrene; (4) coagulant action, transforming prothrombin into thrombin, while others act on the plasma. Observations both *in vitro* and *in vivo* were made on *Lachesis, Crotalus terrificus, Naja* and *Elaps*; the conclusion reached was that as regards their biological actions two types of snake venom exist—Colubrine and Crotaline. The Colubrine type, seen in *Elaps* and *Naja*, acts almost exclusively on the phosphatids and to a much less degree on the proteids. Both have a definite neurotropic action, but differing in the way in which this is exerted. The Crotaline type (*Lachesis* and American *Crotalus*) in addition to a marked action on phosphatids acts powerfully on proteids. *C. terrificus* has marked coagulant effects on proteids, bringing about the disappearance of fibrinogen, but not destroying proteids altogether. The differences in proteolytic effects between *Lachesis* and *C. terrificus* are quantitative rather than qualitative, the former being stronger. The *C. terrificus* of the North has a much more powerful proteolytic venom than that of the South, while all the Crotalines of Central and North America have venoms more potent in this respect than *Lachesis*.

The Anti-Complementary Action of Different Venoms.—VELLARD and VIANNA[12] find that the venoms of the genera *Lachesis* and *Tremeresurus* are anti-complementary, but that their activity in this respect varies with different species. The venom of *Naja tripudians* is much less active, while those of *Crotalus terrificus* and of *Vipera aspis* are not found under experimental conditions to have any action on complement. Considerable differences were observed between the venoms of different species of *Lachesis* and *Crotalus*, and perceptible differences existed between different geographical varieties of *Lachesis neuwiedii*,

[10] SUZUKI (Ch.), MATSUMOTO (K.) & SUGIO (K.). Ueber die Widerstandsfähigkeit der Manguste gegen Schlangengift.—*Taiwan Igakkai Zasshi* (*Jl. Med. Assoc. Formosa*). 1934. Feb. Vol. 33. No. 2 (347). [In Japanese pp. 305–314. With 1 fig. German summary pp. 24–25.]

[11] VELLARD (J. A.) & VIANNA (M. Miguelote). Acção comparada dos diversos venenos ophidicos.—*Rev. Med.-Cirurg. do Brasil.* 1934. Feb. Vol. 42. No. 2. pp. 59–67.

[12] VELLARD (J. A.) & VIANNA (Miguelote). Complemento e venenos ophidicos.——*Rev. Med. Cirurg. do Brasil.* 1933. Oct. Vol. 41. No. 10. pp. 289–322. [11 refs.] French summary.

though they are little marked in those of *Lachesis jararaca* and *Crotalus terrificus*. A certain incubation period is necessary for the action of venom on complement which varies from 15 minutes to 2 hours; some venoms act rapidly, others slowly.

The properties of the venom are not modified after exerting its anti-complementary action, which is due to its proteolytic effect and not to its coagulant properties. Thus the venom of *Naja* is strongly anti-coagulant and non-proteolytic and shows slight anti-complementary tendency; the venom of *V. aspis* only acts on certain albuminoids and does not affect complement at all.

Enzymes in Venom.—DUNN[13] has studied the action of the enzymes of the venom of *Crotalus adamanteus* on the proteins of blood and milk and found that they were all digested.

One or more proteolytic enzymes were present which digested plasma and serum proteins, and with less rapidity serum albumen and globulin; there was also a weak but definite action on rennin. *Crotalus* venom was found to transform haemoglobin into methaemoglobin in solution, but with non-haemolysed corpuscles methaemoglobin could be detected only after slight haemolysis had occurred.

In another paper DUNN[14] reports having separated the enzymes and toxic principles of the venom of *Crotalus adamanteus*. Its ability to destroy cephalin and to digest protein were found to be due to separate constituents. An albumose fraction was prepared which contained cephalinase, but was free from proteolytic activity; a portion of the toxicity of the venom was contained in this fraction which had haemolytic powers of the same order as the original venom. The toxic principles, enzymes and proteins of the venom are all adsorbed by freshly prepared aluminium hydroxide C, while the substance which oxidizes haemoglobin to methaemoglobin is different from proteinase and cephalinase and is easily separated from them.

The Central and Peripheral Action of Snake Venoms.—KELLAWAY[15] continued his studies on the peripheral action of Australian snake venom with special reference to the sensory nerve endings in frogs. The Australian venoms studied were derived from the black snake, the copper-head, the death adder, the tiger snake and the black tiger snake; that of the Indian cobra was also included. All the venoms were found to have a paralysant action *in vitro* on sensory nerve endings in *Hyla aurea*, though this was less powerful than their action upon the motor endings in this species. The arrangement of the venoms in the order of potency was the same for both sensory and motor nerve endings. Curari also paralyses sensory endings *in vitro*, but this action is overshadowed by its much more powerful effect on motor endings.

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[13] DUNN (Edwin E.). The Action of the Enzymes of the Venom of *Crotalus adamanteus* on the Proteins of Blood and Milk.—*Jl. Pharm. & Experim. Therap.* 1934. Apr. Vol. 50. No. 4. pp. 386–392. [12 refs.]

[14] DUNN (Edwin E.). The Separation of the Enzymes and Toxic Principles of the Venom of *Crotalus adamanteus*.—*Jl. Pharm. & Experim. Therap.* 1934. Apr. Vol. 50. No. 4. pp. 393–406. [22 refs.]

[15] KELLAWAY (C. H.). The Peripheral Action of Australian Snake Venoms. 4. Action on Sensory Nerve Endings in Frogs.—*Australian Jl. Experim. Biol. & Med. Sci.* 1934. Dec. 16. Vol. 12. Pt. 4. pp. 177–186. With 3 figs.

VENKATACHLAM and RATNAGIRISWARAN[16] report some experimental observations on the venom of the Indian cobra, and reopen the question whether the respiratory paralysis so produced is due to central or peripheral action. They found that while sub-lethal doses of the venom, so regulated as not to produce respiratory or cardiac embarrassment, paralyse the motor end-plates alone some time after the administration of venom, yet with bigger doses animals die long before paralysis of the end-plates develops. They state that following the injection of large doses of venom in animals, stimulation of the phrenic nerve immediately after death is found to give rise to contraction of the diaphragm, and conclude that lethal doses of Indian cobra venom cause respiratory distress followed by death owing to central respiratory paralysis. The authors make no comment on the most recent experimental work on this subject—notably that of KELLAWAY, who by applying to the phrenic nerve non-polarizable electrodes connected to capacity amplifiers obtained with a loud speaker an audible record of descending motor impulses from the respiratory centre in animals paralysed with colubrid venoms and kept alive by artificial pulmonary ventilation (this *Bulletin*, Vol. 31, p. 99).

Effects of Venoms on the Cardio-Vascular System.—VERNES and KORESSIOS[17] state that in normal people small doses of cobra venom have a hypotensive effect, while in cases of arterial hypertension a fall of blood pressure lasting for some weeks follows the injection of non-toxic amounts of venom. LAIGNEL-LAVASTINE, WÜRMSER and KORESSIOS[18] studied experimentally variations of arterial pressure in the dog, and found a definite hypotensive effect to follow the injection of 1/100–1/50 mgm. of cobra venom per kg. body weight; this hypotensive action persisted after double vagotomy and the injection of atrophine sulphate. After the injection of cobra venom the hypertensive effect of adrenalin was diminished. They concluded that in man the sustained hypotensive action of cobra venom was due to its selective action on the peripheral vessels and was independent of the vagus. GAUTRELET and HALPERN[19] examined frogs injected with cobra venom and showed that during the period of hypotension excitation of the central end of the vago-sympathetic and the sciatic and the application of different substances like adrenalin, choline, nicotine, acetyl-choline and pituitrin indicated that the hypotension was not due to vaso-dilator paralysis. Some evidence in support of the view that there was a direct action on the capillaries comparable to that of histamine was presented. NAKAMURA[20] studied

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[16] VENKATACHALAM (K.) & RATNAGIRISWARAN (A. N.). Some Experimental Observations on the Venom of the Indian Cobra.—*Indian Jl. Med. Res.* 1934. Oct. Vol. 22. No. 2. pp. 289–294. With 1 graph. [14 refs.]

[17] VERNES (Arthur) & KORESSIOS (N. T.). L'action du venin de cobra sur la pression artérielle (homme normal et homme hypertendu).—*Arch. Inst. Prophylactique.* 1934. Vol. 6. No. 1. pp. 20–35. With 14 figs.

[18] LAIGNEL-LAVASTINE, WÜRMSER (Lise) & KORESSIOS (N. T.). Le mécanisme physiologique de l'action hypotensive du venin de cobra.—*Bull. et Mém. Soc. Méd. Hôpit. de Paris.* 1934. Apr. 2. 50th Year. 3rd Ser. No. 11. pp. 494–498. With 6 graphs.

[19] GAUTRELET (J.) & HALPERN (N.). Etude expérimentale de l'action du venin de cobra sur la circulation.—*C. R. Soc. Biol.* 1934. Vol. 115. No. 9. pp. 942–943.

[20] NAKAMURA (Tutomu). Ueber die Wirkung des Giftes der Naja naja atra auf das isolierte Froschherz. II. Mitteilung: Jahreszeitliche Schwankungen der Resistenz des Froschherzens gegen das Kobragift.—*Taiwan Igakkai Zasshi* (*Jl. Med. Assoc. Formosa*). 1934. Feb. Vol. 33. No. 2 (347). [In Japanese pp. 207–211. With 1 chart. German summary p. 17.]

the resistance of the isolated frog's heart to the action of venom of *Naja naja atra* and found the effects varied according to the species of the frog and the season of the year, the toxic effect being accelerated in summer.

GAUTRELET, HALPERN and CORTEGGIANI[21] find that the intravenous injection of 1/20–1/40 mgm. per kg. of *Vipera aspis* venom in the chloralized dog produces an immediate fall of arterial pressure of 50 to 100 mm. which lasts 1 to 2 hours; the pressure takes 4 to 5 hours to return to its original level. Often there is a diminution in the cardiac output within ½ to 1 hour. This fall in pressure is accompanied by dilatation of the peripheral and intestinal vessels, by contraction of the spleen and kidneys and by an increased viscosity of the blood associated with polycythaemia. The latter phenomenon suggests an increased permeability of the capillaries.

CUBONI[22] reports that viperine venom (*Vipera ammodytes*) injected into rabbits intravenously causes an immediate fall in blood pressure, and that specific antiserum when added to the venom inhibits this action; normal horse serum produces the same result, but to a lesser degree. Formalized venom injected intravenously does not affect the blood pressure even when given in large doses.

CHOPRA and CHOWHAN[23] give a detailed account of their work on Indian Daboia venom (*Vipera russellii*) with special reference to its action on the circulatory system. This venom was found to act on the endothelial layers of the blood vessels, particularly the walls of the capillaries, and extensive haemorrhagic phenomena appeared early. There was a marked tendency to produce thrombosis and gangrene at the site of the bite. The systemic blood vessels especially the peripheral ones were contracted, and those of the splanchnic area widely dilated as in histamine shock. The lungs showed haemorrhage, infarction and congestion. The right side of the heart was full of dark blood, there was enormous engorgement of the abdominal viscera and the serous cavities contained much sanguineous fluid. Large doses of venom caused a rapid and permanent fall in blood pressure in both the normal and the decerebrate animal, but when the mesenteric arteries were clamped quite large doses of venom failed to produce any marked hypotensive effect. The fall of blood pressure ordinarily observed could be overcome by pituitrin, adrenalin and large doses of saline. The paralytic action of the venom on the capillaries with increased leakage of fluid into the tissues resembled that of histamine shock, and it was noted that where large doses of histamine were initially injected no further fall of blood pressure followed venom administration. Shock so produced was the main cause of death in Daboia bites.

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[21] GAUTRELET (J.), HALPERN (N.) & CORTEGGIANI (E.). Action du venin de *Vipera aspis* sur la circulation.—*C. R. Soc. Biol.* 1934. Vol. 116. No. 24. pp. 867–868.

[22] CUBONI (E.). Il siero antivipera sopprime l'azione ipotensiva del veleno di vipera.—*Boll. Istituto Sieroterap. Milanese.* 1933. Nov. Vol. 12. No. 11. pp. 841–845. With 3 graphs on 2 plates. [18 refs.] German summary.

[23] CHOPRA (R. N.) & CHOWHAN (J. S.). Action of the Indian Daboia (*Vipera russellii*) Venom on the Circulatory System.—*Indian Jl. Med. Res.* 1934. Jan. Vol. 21. No. 3. pp. 493–506. With 6 figs. [22 refs.]

Effects of Venoms on the Red and White Corpuscles.—VELLARD and MIGUELOTTE-VIANNA[24] studied the venoms of *Lachesis*, *Crotalus* and *Naja* and demonstrated a pronounced lytic effect on red and white corpuscles followed by a stimulating action on the haematopoietic organs characterized by the appearance in the circulation of numbers of immature cells. In poisoning by venom of *Crotalus terrificus* the decrease in the number of red cells was much more evident in the first few hours than with *Lachesis* venom ; the numerical increase of leucocytes on the other hand was more marked and persistent. The venom of *Naja tripudians* was more intensely lytic than either of the others. A delayed polymorphonuclear leucocytosis accompanied gangrene or local abscess formation.

HOLDEN[25] reports on the effects of variations in the concentration of red cells, variations of the hydrogen ion, the presence of trivalent anion and the addition of certain proteins in modifying haemolysis of rabbits' erythrocytes by copper-head venom. It was found that certain proteins had an inhibiting effect, that haemoglobin accelerated haemolysis, and that the hydrogen ion concentration affected the velocity of lytic action.

The Haemostatic Possibilities of Snake Venom.—Acting on the suggestion of Professor H. HARTRIDGE in regard to coagulants in certain snake venoms, MACFARLANE and BARNETT[26] examined the action of various venoms collected from snakes in the London Zoo on haemophilic blood with the view to the production of a haemostatic agent.

Haemophilic blood was obtained from three donors, and to 10 drops of this was added 1 drop of a 1/1,000 solution of the venoms in a mechanical coagulometer, the coagulation time being compared with that of untreated blood. The only genus in which the venoms were consistently coagulant was *Vipera*, and of these *Vipera russellii* yielded the most striking results ; thus in 17 seconds it clotted haemophilic blood which took 35 minutes to clot spontaneously. The deleterious effects of other toxic venom constituents were diluted out by employing a 1/10,000 solution, which provided a clotting time of about 60 seconds—sufficient for all practical purposes—and sterility was ensured by passage through a Berkefeld filter No. 12 bV. The authors state that not enough data have accumulated to allow of definite therapeutic claims, but in both dental and general surgery the solution has been applied with apparent success as a haemostatic in both normal and haemophilic subjects without ill-effects. In genuine haemophilic subjects it has been used most effectively following dental extraction (two cases), to control epistaxis (one case), and to control haemorrhage from wounds (one case). The confirmatory clinical reports will be awaited with great interest.

In a further report on the relative potency of certain snake venoms to coagulate haemophilic blood these authors, BARNETT and MACFAR-

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[24] VELLARD (J.) & MIGUELOTTE-VIANNA (M.). Action de l'envenimation ophidique sur les globules sanguins.—*C. R. Soc. Biol.* 1935. Vol. 118. No. 1. pp. 19–20.

[25] HOLDEN (Henry Francis). Haemolysis by Australian Snake Venoms. 3. Some Factors which influence the Action of the Venom of the Copperhead. —*Australian Jl. Experim. Biol. & Med. Sci.* 1934. June 16. Vol. 12. Pt. 2. pp. 55–61. With 8 figs.

[26] MACFARLANE (R. G.) & BARNETT (Burgess). The Haemostatic Possibilities of Snake-Venom.—*Lancet.* 1934. Nov. 3. pp. 985–987.

LANE,[27] point out that the coagulating ferment is present in many more snake venoms than has been supposed. Its presence in Mamba venom and that of the common Krait are instanced as examples, for here its presence could not have been detected had normal instead of haemophilic blood been utilized in experiments.

Other Observations on Venoms.—BERNKOPF[28] has studied the effects of formaldehyde on the contraction of the isolated uterus of the guinea-pig caused by snake venom, but unfortunately the species is not given. The previous addition of formaldehyde to the Ringer-Dale solution in which the organ is suspended inhibits the uterine contraction which is normally caused by venom. Renewal of the solution is generally followed by contraction when venom or histamine are added. The author is of the opinion that formaldehyde acts directly on the muscle tissue, and that the delayed contraction of muscle occurs when formaldehyde has been washed out of the bath, leaving the venom or histamine free to act. This is contrary to the view of KENDALL, who ascribes the antagonism between formaldehyde and histamine to chemical reaction.

NECHKOVITCH[29] injected cobra venom into the mesenteric and ear veins respectively of dogs, and invariably found that in passing through the liver much of its toxic action was lost. He suggests this is one of the reasons why cobra venom is ineffective when taken *per os*.

PHISALIX and PASTEUR[30] investigated the action of shortwave length radiation on the venom of *Vipera aspis*. Its first action was to destroy antigenic properties and to make the venom more toxic: if adequately irradiated, however, the toxicity was reduced by $\frac{1}{3}$ to $\frac{1}{4}$, the haemorrhagin content of the venom remaining unmodified.

VIII. *Clinical Aspects and Treatment.*

LOUNSBERRY[31] describes a case of rattlesnake anaphylaxis associated with generalized dermatitis. There was a history of having been bitten by a rattlesnake in 1930; the present bite was caused by *Crotalus mitchelli*. Immediately following the bite an itchy, burning, urticarial rash developed: fever, chill and cold sweating followed. Four hours after injection 10 cc. of antivenene were given subcutaneously and two hours later a similar dose intramuscularly. A widespread dermatitis resulted and later developed into a diffuse erythematous papulo-vesicular eruption with blebs forming at certain points, especially around the site of the bite. In 1930 ZOZAYA and STADELMAN had reported a

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[27] BARNETT (Burgess) & MACFARLANE (R. G.). On the Relative Potency of Certain Snake-Venoms to coagulate Haemophilic Blood.—Reprinted from *Proc. Zool. Soc.* 1934. Pt. 4. pp. 977–978.

[28] BERNKOPF (Hans). Ueber die Wirkung des Formaldehyds auf die durch Schlangengifte hervorrufbare Kontraktion des glatten Muskels.—*Ztschr. f. Immunitätsf. u. Experim. Therap.* 1934. Sept. 18. Vol. 83. No. 3/4. pp. 197–203.

[29] NECHKOVITCH (M.). De l'action anticobraïque du foie.—*C. R. Soc. Biol.* 1934. Vol. 115. No. 8. pp. 889–890.

[30] PHISALIX (Marie) & PASTEUR (Félix). Action des ondes courtes sur le venin de vipère aspic.—*C. R. Acad. Sci.* 1934. July 16. Vol. 199. No. 3. pp. 235–237.

[31] LOUNSBERRY (C. Ray). Rattlesnake Anaphylaxis associated with a Generalized Dermatitis.—*Arch. Dermat. & Syph.* 1934. May. Vol. 29. No. 5. pp. 658–667. [18 refs.]

somewhat similar condition in a man who had been inoculated experimentally with venom from *C. mitchelli* and later had been bitten by a copper-head ; subsequently he developed a desquamating, eczematous dermatitis whenever he handled dried venom.

FREY[32] reports that 13 cases of bites by the common adder (*Vipera berus*) were treated in the Königsberg Hospital in 1933 without any deaths. Such procedures as local incision, sucking the wound and bandaging the limb were deprecated. The only reliable treatment was antivenene (Pasteur Institute E.R.) ; 10 cc. of this serum were injected intramuscularly into the neighbourhood of the bite, but if dangerous symptoms supervened the dosage was raised to 40 cc. or more and given intravenously ; in the latter case it is advised to test the patient for hypersensitiveness to horse serum.

GALLI-VALERIO[33] points out that *Vipera aspis* and *Vipera berus*, but not *Vipera ammodytes*, are encountered in Switzerland. The venom yield is approximately 30 to 40 mgm. in each instance and the actions of the venoms are identical. Opinions differ, however, regarding the frequency of lethal effects on man and different authorities are quoted giving variable death rates. In Switzerland from 1817 to 1886 the mortality rate from bites by these vipers was estimated by FREDET to be 7 per cent., while BRENNING more recently calculated the mortality rate for Europe to be 8·5 per cent. PETITPIERRE[34] reviewed 21 cases of venomous snake bite in Switzerland during the past 50 years. The only fatal case was his own : this occurred in a girl aged 10 years who was bitten on the thigh by an adder and brought to hospital about 1¼ hours after being bitten. Antivenene could not be obtained from any chemist in the Upper Engadine and though local measures including ligature, cupping and the local injection of 1 per cent. solution of potassium permanganate were employed the child died within 2 days. He advocates ligature above the seat of the bite and the injection of appropriate antivenene as the most suitable treatment. The four antivenenes available in Europe were discussed—Calmette's serum E.R. prepared at the Pasteur Institute from horses immunized with the venoms of *V. berus* and *V. aspis*, Behring serum prepared by immunization with a number of European and non-European venoms, the Vienna anti-bothrops serum prepared with the venom of *Lachesis jararaca*, which KRAUS has shown to neutralize the venoms of the European vipers, and the Milan serum S.M. made by immunization with the venom of *V. ammodytes*. As only *V. berus* and *V. aspis* are found in Switzerland, PETITPIERRE advocates the use of Calmette's serum.

IX. *Antivenenes.*

PEPEU[35] reports his observations on the specificity of three out of these four viperine antivenenes. Mice were used in these experiments

[32] FREY (Sigurd). Der Kreuzotterbiss.—*Deut. Med. Woch.* 1934. Feb. 16. Vol. 60. No. 7. pp. 240–242. [13 refs.]

[33] GALLI-VALERIO (B.). Observations sur les morsures de *Vipera aspis*, L.—*Schweiz. Med. Woch.* 1934. Aug. 18. No. 33. pp. 773–774.

[34] PETITPIERRE (Marco). Ueber Schlangenbissvergiftungen in der Schweiz mit besonderer Berücksichtigung des Engadins, des Puschlavs und des Bergells.—*Schweiz. Med. Woch.* 1934. Apr. 28. No. 17. pp. 372–380. With 7 figs. (1 plate). [40 refs.]

[35] PEPEU (F.). Recherches sur la spécificité des sérums anti-ophidiens.—*Boll. Sezione Ital., Soc. Internaz. di Microbiologia.* Milan. 1934. Oct. Vol. 6. No. 10. pp. 383–387. [13 refs.]

and the venom and antivenene mixture were kept for 1 hour at 37°C. before intravenous injection. The conclusions reached were that anti-ammodytes and anti-aspis-berus sera neutralized equally all three venoms, that anti-bothrops serum neutralized *V. ammodytes* and *V. lebetina* venoms, but only feebly *V. aspis* venom, and that anti-ammodytes serum can be employed in the treatment of snake poisoning produced by all the European vipers and *V. libetina* of Asia as well. PEPEU[36] studied the venom of *V. ammodytes* to which 0·4 per cent. of formalin had been added and after a variable period of incubation at 38°C. found it was transformed into anatoxin. Dogs were actively immunized with anatoxin prepared from half a gland and subsequently inoculated subcutaneously into the paw or snout at intervals of 15 to 34 days with the venom contained in one gland. Complete protection was found provided the venom was injected subcutaneously, but if given intravenously the animal died.

The keeping properties of antivenenes from 1907 to 1925 have been studied by DO AMARAL, ARANTES and DA FONSECA.[37] They concluded that the precipitate found in antivenenes is composed of pseudo-globulin and does not seem to exert any appreciable influence on their neutralizing activity. Nor does purification of plasma by fractional precipitation of globulin or the hydrogen ion concentration in the ampoules influence its activity after long keeping. Age *per se* is not an apparent cause of inactivity, which occurs early during the first few years and then appears to remain stationary. Once this initial depreciation of titre has occurred its potency generally remains stationary for 25 years at about 50 per cent. of its original value.

PRATT-JOHNSON[38] describes a method of estimating the haemorrhagin content of viperine venom by observing the effects of venom dilutions inoculated intradermally into the depilated skin of albino guineapigs; 0·1 cc. of a series of saline dilutions of dried venom is used, and a fairly sharp end point is reached at which no capillary haemorrhage is produced (negative reaction). The smallest dose of venom which in 30 minutes produces a definite bluish-black area measuring 5 to 10 mm. in diameter is recorded as the minimal skin dose (m.s.d.) for any particular batch of venom.

By mixing falling dilutions of antivenene with a certain skin test dose of viperine venom and after an interval injecting the mixture intradermally the neutralization point is found. Using this technique it is possible to express the potency of the antivenene in terms of its power to neutralize so many minimal skin doses of viperine venom, while the titre of antihaemorrhagin may be observed throughout the course of immunization.

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[36] PEPEU (F.). Essais de vaccination expérimentale anti-ophidienne.—*Boll. Sezione Ital., Soc. Internaz. di Microbiologia.* Milan. 1934. Oct. Vol. 6. No. 10. pp. 380–382.

[37] DO AMARAL (Afranio), ARANTES (J. Bernardino) & DA FONSECA (Flavio). De la durée de l'activité des antitoxines et des antivenins.—*Rev. Sud Américaine de Méd. et de Chirurg.* Paris. 1934. Apr. Vol. 5. No. 4. pp. 209–218. [22 refs.]

[38] PRATT-JOHNSON (J.). The Estimation of Haemorrhagin in Venoms by an Intradermal Method and a Potency Test of Antivenomous Serum for Antihaemorrhagin.—*Jl. Path. & Bact.* 1934. Nov. Vol. 39. No. 3. pp. 704–706.

GREVAL[39] reviews the production of antivenene in India and describes a technique for concentrating the neutralizing factor in the pseudo-globulin fraction. The pseudo-globulin is separated and dialysed after a fractional precipitation of the blood proteins with ammonium sulphate and the dialysate constitutes the concentrated antivenene. The ammonium sulphate method is said to be cheaper, easier and more efficient than the sodium sulphate method recently advocated for use in India by MAITRA, NAIDU and AHUJA (this *Bulletin*, 1933, Vol. 31, p. 104). *N. Hamilton Fairley.**

*Summaries of the German papers were made by Colonel H. J. WALTON.

[39] GREVAL (S. D. S.). Concentration of Antivenene by the Ammonium Sulphate Method.—*Indian Jl. Med. Res.* 1934. Oct. Vol. 22. No. 2. pp. 365–371. With 2 figs. on 1 plate.

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